Vol. 28, No. 4 November, 1941

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

C. T. GIMINGHAM

PUBLICATIONS COMMITTEE

H. G. H. KEARNS F. R. PETHERBRIDGE

R. W. MARSH H. WORMALD

W. C. MOORE

CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)

BOMBAY, CALCUTTA, MADRAS: Macmillan

TOKYO: The Maruzen Company, Ltd.

All rights reserved

PRINTED IN GREAT BRITAIN

The Association of Applied Biologists

President
H. MARTIN, D.Sc., F.I.C.

Vice-Presidents
Miss W. E. BRENCHLEY, D.Sc. F. R. PETHERBRIDGE, M.A.

Hon. Treasurer
W. C. MOORE, M.A.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Editor (General and Botanical)
Prof. W. B. BRIERLEY, D.Sc.
University of Reading,
Berks.

Hon. Editor (Zoological)
C. T. GIMINGHAM, B.Sc., F.I.C.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Secretary (General and Botanical)
W. P. K. FINDLAY, D.Sc.
Forest Products Research Laboratory
Princes Risborough, Bucks.

Hon. Secretary (Zoological)
H. F. BARNES, M.A., Ph.D.
Rothamsted Experimental Station,
Harpenden, Herts.

Council

G. C. AINSWORTH, Ph.D.
G. E. BLACKMAN, M.A.
Miss W. E. BRENCHLEY, D.Sc.
D. E. GREEN, M.Sc.
R. V. HARRIS, B.Sc.
W. E. H. HODSON, D.I.C.
H. G. H. KEARNS, Ph.D.
O. B. LEAN, B.Sc.
E. A. PARKIN, Ph.D.
F. R. PETHERBRIDGE, M.A.
C. POTTER, Ph.D.
W. M. WARE, D.Sc.

CONTENTS OF Vol. 28, No. 4

DAMAGE TO GREENHOUSE PLANTS CAUSED BY TOWN FOGS WITH SPECIAL REFERENCE TO SULPHUR DIOXIDE AND LIGHT

BY C. R. METCALFE

Jodrell Laboratory, Royal Botanic Gardens, Kew

(With Plate 12 and 4 Text-figures)

PUBLICATIONS on the damage caused to vegetation by a smoke-laden atmosphere refer primarily to outdoor vegetation and are cited in *Effect of Sulphur Dioxide on Vegetation* (1939). More recent papers have been published in the United States by Setterstrom & Zimmerman (1939) and in this country by Jones (1940). Damage to ornamental greenhouse plants was studied by Oliver (1891, 1894), and less complete accounts have been published by Falconar (1931), Metcalfe (1937), and in the *Annual Report of the Missouri Botanic Garden* (1941). Oliver's work is mainly descriptive, although he made experiments and suggested possible remedies. In 1935, Sir Arthur Hill suggested a reinvestigation of the subject because the damage was still prevalent in the greenhouses at Kew, and it seemed possible that electrical equipment might be applied to counteract the effect of London fog. The work was done in collaboration with chemists from the Government Laboratory and engineers from H.M. Office of Works. The war brought it to a standstill, but it is desirable to place on record what was done.

Damage to outdoor vegetation has been fully described in the literature cited above. All vegetation may be killed within 50 yd. of a burning pit heap in the direction of the prevailing wind, the foliage of herbaceous crops is liable to be scorched, whilst trees are stunted and often partly or wholly killed in industrial neighbourhoods. Early leaf fall is another symptom, and even privet may become defoliated in winter. Different kinds of plants vary widely in their power of resisting atmospheric pollution: e.g. *Sambucus nigra* is said to be particularly hardy.

Ornamental greenhouse plants show the same type of variation in resistance to a polluted atmosphere as those growing out of doors, but the rationale of the immunity is unknown. Many even of the tropical plants are less easily affected than others, so it is not merely a question of the plants not becoming adapted to the environment in new geographical surroundings. One of the most familiar symptoms is the disarticulation of leaves following a single night's town fog, seen particularly in certain species of *Ruellia*, *Turnera*, *Jacobinia*, *Pelargonium*, *Crossandra*, and above all, in some of the small leaved begonias such as *Begonia foliosa* and *B. fuchsioides* especially the variety *miniata*. In many of the plants the leaves become detached whilst remaining green and apparently healthy; in others they may become scorched before they fall, or alternatively, may remain attached to the plant although scorched and unhealthy in appearance. Symptoms of the last type are more familiar in winter-flowering begonias such as the 'Gloire de Lorraine' varieties. From other winter-flowering plants such as *Plumbago rosea*, *Coleus Frederici* and begonias, the buds become detached without opening, or the fully opened flowers may be lost. Some of the begonias of the 'Gloire de Lorraine' type are more resistant to fog damage than others: e.g. the

varieties 'Rothschild' and 'Ege's Favourite' were less affected during the winter of 1937–8 than were 'Mrs Petersen', 'Cincinnati' and 'Favourite', in which the buds sometimes hardly opened. Orchids, such as the calanthes, are also affected. In these little or no damage is caused until the buds have emerged from the protective covering of the bracts, but subsequent exposure to a polluted atmosphere causes the buds to turn yellow, then black, and finally to fall without opening. Fully opened flowers also become discoloured and die prematurely. The buds of calanthes sometimes turn black for some other unknown reason, particularly in plants with small or poorly developed pseudo-bulbs. Further details about other indoor plants which are liable to be damaged in foggy weather are recorded by Oliver (1891, 1894).

A polluted atmosphere may affect plants in the following ways: First a sticky, adherent scum of tarry substances is often deposited on the foliage, thereby reducing the light intensity available for photosynthesis, or solid deposits may also become lodged in the stomata and so slow down the gaseous exchange between the leaf and the surrounding atmosphere. Both of these types of damage apply especially to outdoor plants, notably broad-leaved evergreens and conifers which are frequently weakened and stunted. Solid atmospheric impurities are usually less harmful to greenhouse plants since these are not so fully exposed to their action, but sooty deposits on the glass may still further reduce the intensity of winter daylight which is often too low to maintain exotic plants in a good state of health. Secondly, plants are affected by the direct toxic action of chemical substances. Invisible gases such as ethylene are particularly harmful even in small doses, and this constituent of household gas may cause damage in certain districts. Compounds of iron, arsenic and other substances in the atmosphere may also account for some of the harm, but it is shown below that acid sulphur compounds, of which the most important is SO_2, constitute one of the main sources of damage to glasshouse plants at Kew. All of these toxic substances are liberated in the air when coal or smokeless fuels such as coke, are burned. These investigations have also shown that, although reduced illumination is less important than the presence of toxic substances, it may have a weakening effect on the plants.

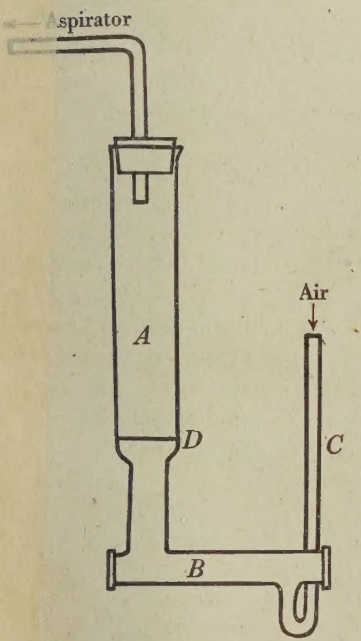
Three main types of investigation have been made with SO_2. (i) The SO_2 content of the air was examined, and an attempt made to correlate the amount of damage done with the extent of the pollution. (ii) The effect of very low concentrations of SO_2 on the plants under different conditions was studied. (iii) Attempts were made both in the laboratory and in the greenhouse to find means of removing SO_2 from the air, and to determine whether air purified by these methods is harmless to plants.

(i) Estimation of the sulphur dioxide in the atmosphere

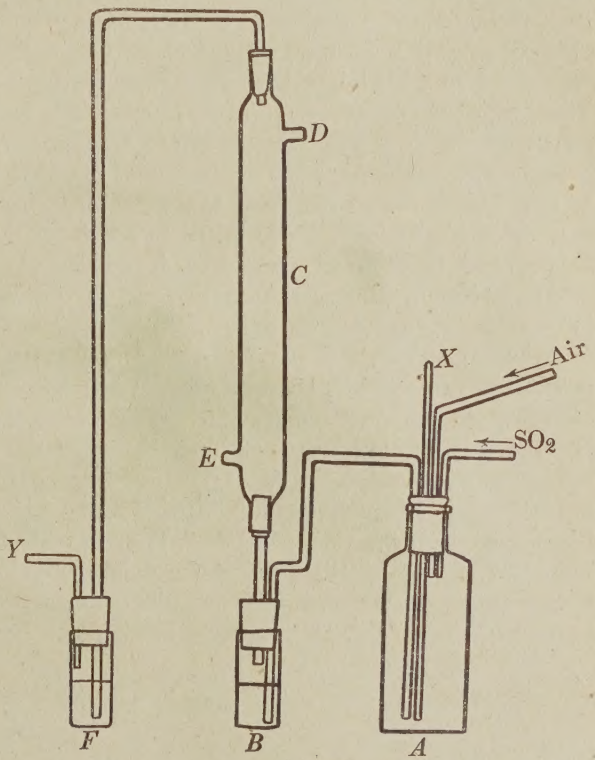
The sulphur content of the atmosphere over various towns has been repeatedly determined in recent years. It is definitely established that the concentration sometimes reaches 0·5 p.p.m. in the London area, and it may well be higher in the industrial North. In many instances, however, these determinations have been made by estimating the quantity present over comparatively long periods, thus giving values for the average concentration in different districts throughout the year. This fails to give a clear picture of the variations in concentration which occur in any one position from hour to hour, or of those in different positions within the confines of a single large garden. It seemed probable that a local high concentration of short duration might often cause damage in greenhouses, so a simple, roughly

quantitative method was devised with which to determine the concentration of SO_2 in different parts of the gardens and in different greenhouses. Oliver (1891) did the same thing by assuming the volume of air which decolorized a solution of potassium permanganate of known volume and concentration, when aspirated through the liquid, to give a rough indication of the SO_2 pollution. During the present investigation Mr B. A. Ellis and Mr R. A. Jones of the Government Laboratory developed a similar but more delicate method.

A mixture of starch and iodine solutions was prepared so as to produce a standard tint of blue in a special apparatus (Text-fig. 1) through which air was aspirated until the liquid became decolorized. The volume of air required to decolorize the liquid was taken to be roughly in inverse proportion



Text-fig. 1. Apparatus for investigating the SO_2 content of the atmosphere. For description see text (p. 303).



Text-fig. 2. Apparatus for removing SO_2 from air. For description see text (p. 309).

to the concentration of SO_2 in the atmosphere. No attempt was made to determine the actual quantity of SO_2 by this method, but it enabled comparisons of the atmosphere to be made at different times and in various parts of the gardens on a single day, as well as at different times of the year.

The broad vertical tube *A* in Text-fig. 1 was connected to an aspirator. The horizontal tube *B*, exactly 10 cm. long, was sealed at either end by means of a glass disk. *C* is a narrow tube through which atmospheric air is drawn into the apparatus by means of the aspirator. The mixture of starch and iodine solutions was introduced through the broad upper end of *A* until the level was brought up to *D*. The concentration of the starch/iodine mixture was adjusted so that the blue colour in *B* exactly matched '18 blue' on Lovibond's scale when viewed through the glass disk at either end of

the tube. Air was then aspirated through the apparatus as quickly as possible until the blue colour had completely disappeared from the liquid in *B*. An inverted T-shaped tube, having the horizontal part the same size as *B* in Text-fig. 1, was filled with a similar standard tinted solution and used for comparison, thus ensuring that any colour change was caused by the aspirated air.

A 0·05% solution of Lintner's starch was used. The iodine solution was at an approximate concentration of *N*/12,500. This was prepared by diluting a *N*/10 solution to *N*/2000, the final dilution being in water containing about 2 g. of potassium iodide/l. Further dilution was made with a solution containing 2 g. of potassium iodine and 0·1 g. of sodium bicarbonate/l. Equal volumes of the starch and iodine solutions when mixed gave a blue colour of approximately the right tint, the colours being finally matched by eye.

Some of the numerous readings taken with this apparatus are shown in Table 1. Little or no decolorization was effected by aspirating 50 l. of air through the apparatus during clear weather in either winter or summer. It was therefore decided to regard the atmosphere as 'free' from SO_2 when 50 l. failed to produce a detectable change. The results roughly correspond to what might have been expected from the appearance of the atmosphere. Thus, whilst the concentration of SO_2 is definitely greater in misty than in clear weather, it is higher still during smoky or yellow fogs. Secondly, although the atmosphere in a well-constructed greenhouse is less polluted with SO_2 than it is out of doors, the tests made on 20 Dec. in greenhouse 18 (Table 1) show that even here it becomes polluted, particularly near the roof. At lower levels in the house the SO_2 may tend to be absorbed on moist surfaces and thereby made more difficult to detect.

The maximum damage to plants was observed when less than 10 l. of air decolorized the solution in outdoor conditions. When the value was above 10, slight damage was sometimes observed. This shows that the seriousness of the damage to the plants is roughly proportional to the SO_2 concentration. Preliminary tests also indicated that reasonably consistent results were obtained in standard conditions. If one compares the readings obtained in an orchid house on 24 June, when there was no fog, with one of those determined in the same greenhouse on 21 Oct. when the weather was foggy (Table 1), there is not much difference in the values. This may mean that SO_2 was penetrating this particular house from the boiler via the hot water pipe channels, when the summer reading was taken. On 1 Nov. the same orchid house seems to have been less polluted than on 21 Oct., possibly because an air purifier with inefficient filters (see section on air purification) was operating in the house on 21 Oct., but not on 1 Nov.

(ii) Experimental production of symptoms similar to those which occur during or after a fog

The following experiments, all repeated many times, were intended to induce symptoms similar to those which are caused by town fog. Plants or cut shoots of *Begonia foliosa* and *B. fuchsioides* var. *miniata* were used except where stated.

Exp. 1. An assortment of susceptible plants was treated with an oleum mist at the end of November by placing fuming sulphuric acid in an open dish and distributing it throughout the greenhouse with an electric fan. The atmosphere meanwhile was kept moist by spraying at intervals with a syringe, so that the acid mist was maintained for 5–6 hr. Although the atmosphere became unpleasant to human beings, very few leaves became detached. This may have been because the physical conditions were in some way unsuitable for the operation of the acid.

TABLE 1. *Volume of air required to decolorize a standard tinted starch/iodine mixture under different conditions*

Date	Locality	Weather conditions	Vol. of air (l.) required to decolorize the solution	
23. vi. 37	Laboratory	Fine	50	Colour almost unchanged
	*Near lamp burning 0·1 % thiophene in methylated spirit	,,	16	Completely decolorized
	,, ,, ,,	,,	17	,, ,,
	,, ,, ,,	,,	16	,, ,,
	,, ,, ,,	,,	18	,, ,,
	,, ,, ,,	,,	17	,, ,,
	,, ,, ,,	,,	16	,, ,,
	An orchid house near chimney	,,	32	,, ,,
24. vi. 37	Outside laboratory	,,	50	Very slight decolorization
	In the gardens	,,	50	,, ,,
	An orchid house	,,	30	Almost decolorized
	,, ,,	,,	50	Partly decolorized
18. x. 37	Outside laboratory	Foggy	17	Completely decolorized
21. x. 37	,, ,, 8.30 a.m.	,,	18	,, ,,
	,, ,, 9.45 a.m.	,,	12	,, ,,
	An orchid house	,,	34	,, ,,
	A greenhouse	,,	50	Nearly decolorized
1. xi. 37	Outside laboratory, 9.30 a.m.	Thick mist	22	Completely decolorized
	,, ,, ↓	,,	17	,, ,,
	,, ,, 10.30 a.m.	,,	19	,, ,,
	An orchid house	,,	50	Blue tinge still remaining
26. xi. 37	Outside laboratory, 9.30 a.m.	,,	25	Completely decolorized
	,, ,, ↓	,,	18	,, ,,
	,, ,, 10.30 a.m.	,,	20	,, ,,
27. xi. 37	,, ,, 9.30 a.m.	Very smoky fog	3	,, ,,
	,, ,, ↓	,,	15	,, ,,
	,, ,, ↓	,,	12	,, ,,
	,, ,, 10.30 a.m.	,,	15	,, ,,
	,, ,, 11.30 a.m.	,,	11	,, ,,
	An orchid house (air conditioned)	,,	50	Not quite decolorized
20. xii. 37	Outside laboratory 10.0 a.m.	Severe fog	9	Completely decolorized
	,, ,, ↓	,,	7	,, ,,
	,, ,, ↓	,,	6	,, ,,
	,, ,, 11.0 a.m.	,,	6	,, ,,
	Greenhouse 18 well constructed, 11.30 a.m.	,,	45	Not quite decolorized
	Greenhouse 18 near roof, 12 noon	Fog clearing	30	Completely decolorized
	Greenhouse 18 near roof, 1 p.m.	,,	45	,, ,,
21. i. 41	Greenhouse 9.0 a.m.	Smoky fog and rain	40	,, ,,
	,, ↓	,, ,,	45	,, ,,
	Outside laboratory ↓	,, ,,	9	,, ,,
	,, ,, ↓	,, ,,	10	,, ,,
	,, ,, ↓	,, ,,	9	,, ,,
	,, ,, ↓	,, ,,	9	,, ,,
	Greenhouse 11.0 a.m.	,, ,,	18	(door open for about 1 hr. since previous greenhouse reading)
	,, 2.30 p.m.	Fog cleared	50	Only partly decolorized

* Air aspirated from below an inverted tin fixed about 2 ft. above the flame.

Exp. 2. A plant of *B. foliosa* was treated by means of a throat spray with dilute sulphuric acid made by adding 2 drops of a 30% solution to about 20 c.c. of water. A similar plant was sprayed with a solution of carbolic acid made by adding a few crystals to about 20 c.c. of water. After 24 hr. only two leaves had fallen from the plant sprayed with carbolic acid, but a considerable number had become detached from that sprayed with sulphuric acid.

Exp. 3. Plants or cut shoots of *B. foliosa* were placed under bell jars together with small dishes of very dilute sulphurous acid, prepared by passing a small quantity of SO_2 into water. Other specimens were similarly treated but the sulphurous acid was replaced by clean tap water. A third group, also accompanied by tap water, was kept in the dark by covering the bell jars with black paper. All of these treatments were repeated at various times of the year and always gave similar results. A few leaves fell from the plants either in light or darkness when accompanied only by water, but a trace of SO_2 accentuated the effect so much that the specimens were often completely defoliated after a single night. The leaves which fell usually remained green and healthy, just like those which drop after a night's fog.

Similar experiments with H_2SO_3 caused the buds and flowers to fall from begonias of the 'Gloire de Lorraine' type, whilst the leaves became scorched but seldom detached: SO_2 applied in this way also produced unhealthy symptoms, similar to those which occur in foggy weather, in *Calanthe* flowers and buds. A higher concentration of H_2SO_3 and a higher temperature were necessary to induce the unhealthy symptoms described in 'Gloire de Lorraine' begonias and calanthes than was necessary to defoliate *Begonia foliosa*. It will be noted that in all of the above instances the SO_2 was operating under very humid conditions.

Exp. 4. Plants and cut shoots were treated with known low concentrations of SO_2 comparable with those which occur in the atmosphere during a fog. The new method employed for this purpose was devised by Mr B. A. Ellis and Mr R. A. Jones of the Government Laboratory, who also constructed and calibrated the apparatus (Pl. 12).

The principle of this apparatus is that a small but known quantity of SO_2, obtained by burning a solution of thiophene in methylated spirit, is mixed with a current of air of known velocity, the mixture then being passed over a suitable plant or cut shoot as a test object. Here again *Begonia foliosa* and *B. fuchsioides* var. *miniata* were the plants most frequently employed.

The air current was generated by the electric blower *A*, any lubricating oil which came over with the air being removed by passing it through empty bottles and finally a U-tube containing cotton wool. The velocity of the air was determined by passing it through the Venturi gauge *B*, after leaving which the air passed under a bell jar *C* standing with its base immersed in water. A broad funnel-shaped tube *D* was fixed with its narrower end in the corked neck at the top of the bell jar *C*, whilst a specially constructed metal lamp *E* with a wick made of glass capillary tubes was placed beneath the wide lower end. A 0·1% solution of thiophene in methylated spirit was burned in the lamp. By weighing the lamp before and after the experiment it was possible to calculate the weight and hence the volume of SO_2 in a given time. It was necessary to keep the lamp immersed in cold water, otherwise the heat generated under the bell jar caused the methylated spirit to expand and overflow, thereby starting a fire. If the lamp burned at the rate of 5 g./hr., whilst the air was flowing at the rate of 1000 l./hr., the air mixture passing out from beneath the bell jar would contain 1 p.p.m. of SO_2. It was found in practice to be easiest to control the concentration by maintaining the air current at constant velocity and to vary the rate of combustion and concentration of the thiophene. The air with its low content of SO_2 was next passed over the experimental plant in the bell jar *F*, beneath which there was also a thermometer. The excess of air and SO_2 then escaped round the loosely fitted joint where the tube leads into *F*. The experimental plant or cut shoot was always arranged with its basal end passing through a hole in a glass plate on which the bell jar was standing, thus ensuring that only the aerial part of the plant was exposed to the SO_2. The temperature under the bell jar beneath which the plant was treated was about 60–70° F.

The symptoms induced by the various treatments were noted about 24 hr. after the beginning of each test, except where stated. The early tests showed that passing a current of air at the rate of 1000 l./hr. for 6 hr. whilst the lamp was burning methylated spirit without thiophene caused no defoliation, provided the atmosphere was free from fog and the plant or shoot thoroughly healthy. If the atmosphere was foggy, or the plant had been weakened by a previous experiment, partial defoliation sometimes occurred. Exactly comparable tests with the addition of thiophene to the lamp, so as to give the begonia a 6 hr. treatment with air containing 1 p.p.m. of SO_2 or very slightly more, also caused partial defoliation, but the effect was not so complete as after a severe fog. It seemed probable that defoliation was incomplete because the treatment was not long enough or the atmosphere beneath the bell jar insufficiently humid.

Exp. 5. This was conducted on the same lines as Exp. 4 except that a Petri dish of water with cotton wool partly immersed in it was placed in *F* beside the experimental plant or cut shoot, and, at the end of the treatment, the top of the bell jar was sealed with a sheet of cellophane held in position with a rubber band. The tube leading from *C* to *E* was disconnected before the cellophane cap was fixed. The duration of the treatment was 2–6 hr. in different instances, but it was, of course, possible for the SO_2 to operate on the plant in a very humid atmosphere after the air current had been stopped.

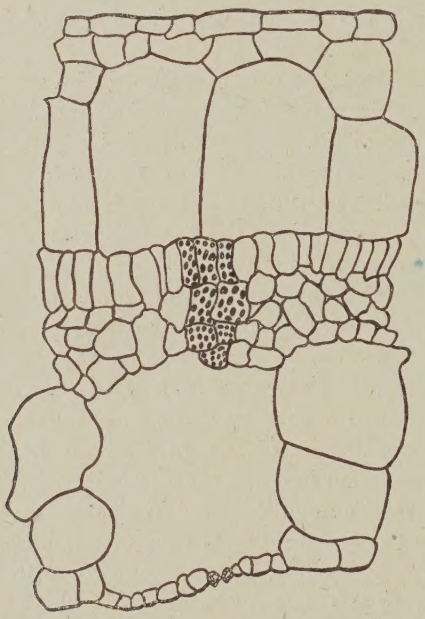
It was shown repeatedly that a 6 hr. treatment at a concentration of about 1·1 p.p.m. of SO_2 caused complete defoliation. Two separate treatments of 1 hr. each were equally effective. Even with a 1 hr. treatment at a concentration of 0·25 p.p.m. or even less the leaves fell off, although the damage was less severe. If a plant was only partly defoliated at the end of the first 24 hr., it, nevertheless, continued to shed leaves on consecutive days. This set of experiments showed a very low concentration of SO_2 in a humid stagnant atmosphere to be more toxic than a higher concentration in a current of relatively dry air.

Exp. 6. This was similar to Exp. 5, but the water and cotton wool in *F* were replaced by anhydrous calcium chloride. In these conditions a concentration of 1 p.p.m. administered for 2 hr. caused only a very few leaves to fall even after 48 hr. Other treatments with SO_2 at various concentrations of the order of 1 p.p.m. or less were made on shoots which were not accompanied by water and cotton wool or by calcium chloride. In these the reduced humidity seemed to render the SO_2 less toxic, but at the same time more damage was caused than when calcium chloride was present.

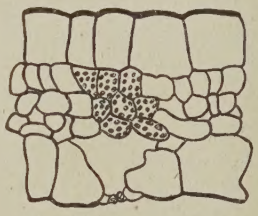
Exp. 7 (*control tests*). A final series of control tests on similar lines to Exp. 5, but in which methylated spirit was burned without the addition of thiophene, failed to cause defoliation, or at most caused only a very few leaves to fall. Those parts of the apparatus in which SO_2 had been present in previous experiments had to be washed thoroughly in running water before consistent results could be obtained. Also a lamp had to be used in which no thiophene had ever been burned. Without these precautions, traces of SO_2 adhering to the inside of the glass apparatus, or produced by remnants of thiophene in the lamp, caused a certain amount of leaf-fall. This, in itself, demonstrates the considerable toxicity of the gas in appropriate conditions.

Since SO_2 dissolves freely in water, it is conceivable that the concentration in these experiments may have increased by cumulative absorption to a value considerably above that which was theoretically being administered. This may also apply in greenhouses where the gas will tend to be absorbed by moisture on plant surfaces, or even within the

cells of the leaf. For this reason it is quite probable that defoliation or other unhealthy symptoms are actually caused by higher concentrations than appears to be the case at first sight. It is interesting to note in this connexion, that most of the thickness of the leaf of *Begonia foliosa* is made up of large water-storage cells, with intercellular spaces between them, the chlorenchyma being confined to a relatively small tissue at the centre (Text-fig. 3). A leaf of this kind might be particularly well adapted to absorb SO_2. The leaves of the more resistant 'Gloire de Lorraine' begonias are similar in structure, but the water-storage cells are smaller (Text-fig. 4).



Text-fig. 3. Transverse section of leaf of *Begonia foliosa*, showing the large water storage cells and intercellular spaces towards the outside, and the narrow band of relatively small-celled chlorenchyma at the centre. This type of *Begonia* is particularly susceptible to damage by SO_2. ($\frac{1}{6}$ objective, no. 2 eyepiece.)



Text-fig. 4. Transverse section of leaf of *Begonia*, variety 'Gloire de Lorraine'. The water storage cells and intercellular spaces are smaller than those of the more susceptible *Begonia foliosa* shown in Text-fig. 3. ($\frac{1}{6}$ objective, no. 2 eyepiece.)

(iii) Experiments to test the protective value of paper coverings against SO_2

It is a horticultural practice in some places to protect plants against the action of a town fog by covering them with newspaper. The two experiments which follow were intended to test the value of this practice for reducing SO_2 damage. The species used were *B. foliosa* and *B. fuchsioides* var. *miniata*.

Exp. 1. A cut shoot under the bell jar *F* in Pl. 12 was covered with an inverted, wide meshed, wire basket, placed inside the bell jar. A paper bag was placed over the wire basket like a hat on a wearer's head, and a dish of water and cotton wool included beside the cut shoot underneath the cage and paper bag. Following a 4 hr. treatment at a concentration of 1·5 p.p.m. the bell jar was sealed with cellophane. After 24 hr. the same shoot was given a second treatment at a concentration of 1·23 p.p.m.

During the 24 hr. following the first of the above treatments very few leaves fell, and those which did were from the basal end of the shoot which was less effectively protected by the paper bag. After the second treatment most of the leaves became detached in spite of the protection provided by the bag.

Exp. 2. This was on similar lines to Exp. 1, but the begonia shoot was replaced by litmus paper. One piece was suspended inside, and a second piece outside the bag but inside the bell jar. A 3 hr. treatment with SO_2 at a concentration of about 1·5 p.p.m. was administered, the apparatus then flushed with clean air and the litmus papers examined. A second SO_2 treatment was administered after fresh litmus paper had been suspended outside the paper bag and the original blue piece replaced within. The bell jar was then sealed with cellophane for 17 hr. before the litmus papers were examined again.

The outer litmus paper turned red quite quickly in both instances, but the inner one remained blue. This demonstrated the protective value of the paper bag, and indicated that the gardener is using a sound practice in covering his plants with newspaper as a means of protection.

(iv) Removal of sulphur dioxide from the atmosphere by filtration, and its application to air-conditioning greenhouses

The most successful of the various methods which were tried for removing SO_2 from the air was by passing it through a column of the spongy moss *Sphagnum* which had been previously dried and soaked in a solution of sodium carbonate. This was done on a laboratory scale and then in certain greenhouses.

The central part of the apparatus used in the laboratory is shown in Text-fig. 2. SO_2 from a siphon was bubbled slowly through a tube containing a little mercury, passing thence into *A* where it was mixed with air from a blower. The mixture of air and SO_2 was then conducted into the glass tube *B* containing litmus solution, passing then into *C* which is the outer part of an ordinary Liebig's condenser filled with *Sphagnum* soaked in a 20% solution of sodium carbonate.

In the early experiments a continuous downward trickle of the alkaline solution was maintained in *C*, by allowing it to enter at *D*, but subsequently this was found to be unnecessary because the *Sphagnum* retained sufficient liquid to neutralize the acid during a long period. Waste solution escaped at *E*. After passing through the column of *Sphagnum*, the purified air was conducted into a tube *F* containing litmus solution. By comparing the colour of the litmus solution in *B* and *F* respectively it was possible to determine whether the SO_2 was removed during its passage through *C*. No attempt was made to determine the concentration of the SO_2 in the mixing chamber, but it was always very much higher than it is ever likely to be in a natural foggy atmosphere.

All of the tests demonstrated the great efficiency of the alkali-soaked moss in removing SO_2 from the current of air. On one occasion the apparatus was run continuously from 10 a.m. to 4.30 p.m., but the litmus solution in *F* remained blue, whilst the similar solution in *B* turned red almost as soon as the experiment was started. For another test the apparatus was connected at *X* and *Y* to bell jars under which there were cut shoots of *Begonia fuchsioides*.

The shoot in the jar connected to *X* was treated with the mixture of air and SO_2 for a period of 10 min., after which the bell jar was disconnected from *X*, sealed with a sheet of cellophane at the upper end and left for observation. The purified air continued to be passed from *Y* into the second bell jar for about 4 hr., after which this one was also disconnected and sealed. The concentration of SO_2 was so strong that the leaves of the begonia which had been treated with the unpurified mixture began to look unhealthy within ½ hr. At the end of a few hours the leaves were yellow and wilted, and by the following morning the shoot was dead. The begonia which had been treated with the purified air remained healthy.

In another experiment, air from *X* and *Y* respectively was passed into a starch and iodine mixture, the colour of which matched '18 blue' on the Lovibond scale. A few cubic centimetres of the unfiltered air from *X* were sufficient to decolorize the solution completely, whilst 50 l. of the purified air from *Y* failed to produce any appreciable colour change. The great sensitivity of the starch and iodine mixture to SO_2 clearly proved the efficiency of the filter.

Other tests showed a shorter column of *Sphagnum* to be sufficient to remove the SO_2 during a period of about 4 hr., but after this a certain amount of SO_2 was let through. As soon as a fresh supply of the sodium carbonate solution was added to the *Sphagnum* the efficiency of the filter was restored.

In certain experiments with the same apparatus, the *Sphagnum* and sodium carbonate solution were replaced by dry activated charcoal, of the type commonly used in gas masks. This was found to be completely ineffective in removing SO_2 from the air current. Other tests in a different apparatus also demonstrated the inefficiency of various filters made of fabric materials even when these were soaked in a solution of sodium carbonate. It is necessary to expose the polluted air current to a very large surface of the alkaline liquid to ensure complete extraction of the acid gas.

In collaboration with H.M. Office of Works, attempts were made to use the *Sphagnum* and alkali method of purification in certain greenhouses.

For this purpose metal units were designed which could be filled with *Sphagnum*. This was well soaked in sodium carbonate solution, and kept moistened with the same liquid by allowing it to trickle from a tank, connected by a pipe to the main unit. The apparatus was fixed outside the greenhouses, but opened into the interior via the substage ventilators. Electric fans sucked air from outside the greenhouses through the *Sphagnum*, after which it passed through a fabric filter to remove solid particles before finally entering the house over hot water pipes.

It is impossible to draw final conclusions concerning the practical value of these units, but the results are promising. It is necessary to have a greenhouse wholly devoted to experiments on these lines, so that adequate controls can be arranged for comparison.

In this connexion Oliver described a fog-filtering device designed by a Mr Toope. In Toope's apparatus the air current was maintained by means of suitably arranged hot water pipes, which, by raising the temperature near the roof of the greenhouse, served to draw cold foggy air inwards through boxes of charcoal which served as filters. Oliver was apparently favourably impressed by this device, but in the light of the present work it seems dubious whether the charcoal was a really efficient filter.

(v) The use of chemicals to counteract the effect of sulphur dioxide

Attempts to reduce the damage caused by SO_2 by spraying plants with alkaline solutions proved to be dangerous because the solutions themselves were harmful to the plants. When dishes of 2% ammonia solution (880 ammonia) were placed in the houses in the hope that the ammonia gas might neutralize the SO_2, the gas diffused away too quickly to be effective, or, when more concentrated, caused more harm to the plants than the fog which it was intended to counteract. The same difficulties were also experienced with ammonia produced by the decomposition of solid ammonium carbonate. These experiments confirmed Oliver's opinion that the use of chemicals for countering fog has nothing to commend it.

(vi) The use of electric light to supplement daylight

Although toxic substances have turned out to be the primary cause of many of the unhealthy symptoms exhibited by plants in foggy weather, the inadequate daylight must doubtless have a weakening effect as well in some localities. Experiments with supplementary illumination from artificial sources were accordingly started, but it is proposed only to record in outline what has been done because this work was interrupted by the outbreak of war.

Previous workers had sometimes found high power filament lamps to be unsuitable for plant irradiation because so much of the electrical energy which they consume is liberated in the form of heat, which tends to 'draw' the plants. Running costs are also high in proportion to the light intensity because so much energy is wasted. At the same time this source of light is cheap to instal and the visible radiation has a comparatively wide spectrum range. With neon discharge lamps a higher proportion of the electrical energy is liberated in the form of visible radiation, most of which is in the red/orange part of the spectrum and therefore valuable for promoting photosynthesis. Neon lighting is more expensive to instal but cheaper to run than filament lamps. Its value for plant irradiation has been demonstrated by Roodenburg (1930–7). It was also thought that a further improvement might be made by combining neon with another source of light in order to increase the spectrum range. This subject was discussed with Prof. V. H. Blackman, and subsequently with Mr C. C. Patterson and members of his staff at the Research Laboratories of the General Electric Co., Ltd., with the ultimate result that the third type of irradiator described below, in which neon and mercury vapour lights were used together, was designed and constructed by the G.E.C. The effect of this combination of light sources on plants does not appear to have been investigated previously, although Arthur & Harvill (1937) used sodium vapour and mercury arc lamps together for certain experiments.

The following sources of light were eventually used: (1) Low power tungsten filament lamps with suitable reflectors arranged parallel to and a few feet above the benches to be illuminated. (2) A 475 W. neon irradiator provided with a suitable reflector, suspended some 5 ft. above the plants: this equipment effectively irradiated about 50 sq. ft., but its influence extended considerably beyond this range, so that the plants included in an area of about 75 sq. ft. were affected. (3) A 400 W. neon lamp combined with four 250 W. 'Osira' high pressure mercury vapour lamps, the former in a rectangular reflector and the latter each in a dispersive reflector. The ratio of mercury vapour to neon illumination given by this combination was stated by the makers to be 4 to 1 over quite a large area. This irradiator was also suspended about 5 ft. above the plants, and effectively irradiated about the same area as the neon light alone.

Owing to the different wave-length qualities of these sources of light their intensities are not strictly comparable. The intensity on a standard surface at various levels beneath each source of light was measured by two independent observers with a Weston Holophane Lumeter, kindly loaned by Prof. V. H. Blackman, so that the conditions could be standardized in separate experiments. The intensities were as follows:

Type of light	Position in relation to the greenhouse bench	Intensity (f.-c.)
Neon	Bench level	25
	14 in. above the bench	70
	3 ft. above bench	150
40 W. frosted filament lamps	Bench level	10
	14 in. above bench (more intense at back of bench)	22–60
Neon and mercury vapour	Bench level	180
	16 in. above bench	230
	3 ft. 6 in. above bench	650–700

The daylight intensities at bench level, measured with the same lumeter in different greenhouses on a fairly bright November afternoon ranged from 200 to 370 f.c. The lamps were turned on from 8 a.m. to 8 p.m. on dull days during the winter months; from dusk until 8 p.m. when the weather was reasonably bright; or during dull periods on days when the natural illumination was variable.

This treatment was intended to ensure that the plants received 12 hr. illumination of reasonable intensity each day. The plants chiefly used in these experiments were begonias of various kinds, but especially the 'Gloire de Lorraine' varieties, calanthes, *Coleus Frederici*, *Euphorbia fulgens*, various cinerarias and a few hyacinths. The most important points which have emerged so far are as follows:

Artificial light does not appreciably inhibit the disarticulation of buds, flowers or leaves of begonias, *Plumbago rosea* and *Coleus Frederici* during foggy weather. Nor does it prevent the appearance of unhealthy symptoms in calanthe buds and flowers. This confirms that these symptoms are due primarily to toxic substances in the atmosphere rather than to insufficient daylight.

Earlier flowering is promoted in cinerarias, hyacinths and winter-flowering begonias. In some instances the duration of the flowering period of the begonias was also prolonged. The flowering period of *Euphorbia fulgens* is hastened but not prolonged, and when flowering is over, vigorous young vegetative shoots develop very rapidly on the irradiated plants. The general healthiness of the begonias of the 'Gloire de Lorraine' type was much improved by neon light, the foliage assuming a dark green colour, and, in at least one instance, the average and maximum diameter of the flowers was greater than in similar plants in a neighbouring greenhouse where there was no electric light. *Coleus Frederici* flourished under neon light, but it is necessary to cultivate this species at a high temperature in a well constructed house to attain good flowering and avoid the premature shedding of buds.

Low power tungsten filament lamps were not satisfactory for the irradiation of winter-flowering begonias, which become somewhat drawn up and sickly in appearance, whilst the flowers were very poor. Comparable plants which were not irradiated were definitely superior.

Neon light alone or neon light combined with mercury vapour gave more satisfactory results than the tungsten filament lamps with all of the plants on which experiments were made. The neon and mercury vapour light combination did not appear to produce a more beneficial effect than the neon light alone in the particular circumstances in which the experiments were conducted. The plants were more unsightly to look at whilst they were being irradiated with the neon light alone, owing to the quality of the light. Whilst being treated with neon and mercury vapour light together, the appearance of the plants and flowers was natural. This point might be important if the plants were open to public exhibition whilst being irradiated.

(vii) Remedies

The most elementary precaution is to render the greenhouses as air-tight as possible. This is not always so easy as might be expected, partly because doors have to be opened and shut in all weathers, and also because of leakages round doors and badly fitting ventilators. Gaps between overlapping panes of glass are often deliberately left for the escape of condensed moisture, in the belief that water will drip from the roof unless they are present. The Palm House at Kew is badly designed from this point of view, and considerably more damage

occurs there than in the new glasshouses where there is less leakage. Filling the cracks between overlapping panes of glass with putty sometimes has a very beneficial effect on the plants, but does not prevent damage altogether, especially when fogs are prolonged or dense.

Boiler house chimneys should be so placed that the prevailing wind blows the flue gases away from the houses, whilst the tops of the chimneys should be as high as possible. Some kinds of solid fuel emit more SO_2 than others, e.g. anthracite often emits less than coke. Oil fuel is also advantageous from this point of view.

A limited number of particularly valuable plants can sometimes be saved by protecting them with paper coverings.

The temperature and humidity should be kept as low as possible for the particular plants concerned. Some evidence has been obtained that keeping the air in motion by electric fans is somewhat beneficial during foggy weather, probably because they help to lower the temperature and promote evaporation.

Fans may also be used to introduce filtered air from outside, although polluted air will still be able to gain access to the houses elsewhere. It will therefore be necessary to introduce sufficient purified air into the house to raise the pressure to slightly above that outside, and so to reduce the leakage inwards of polluted air. Even if SO_2 cannot be completely excluded by this method, it will dilute it considerably with purified air. An air-conditioning system of this kind should give the best results, but it may prove difficult to instal in greenhouses which already exist, although it ought to be possible to build greenhouses suitable for air-conditioning.

Allowing ammonia to diffuse into the atmosphere in greenhouses, or spraying alkaline solutions on the foliage, either failed to prevent damage by SO_2, or caused more harm than the acid which they were intended to counteract.

No single infallible remedy is available, and the most that can be done is to apply common-sense methods which suggest themselves in the light of the fact that SO_2 is one of the principal causes of the damage. The primary essential is to exclude this substance from the houses, or to reduce the toxicity by suitably controlling the temperature and humidity. Supplementary artificial light is beneficial when the fog is sufficiently black to cause serious reduction of photosynthesis, or to render the plants more susceptible to damage by SO_2. It is also desirable to ensure that the plants are adequately provided with mineral nutrients.

Discussion

The failure of an oleum mist to produce such pronounced leaf fall in begonias compared with the much greater effect caused by very low concentrations of SO_2 may have been in some way connected with the physical conditions in which the experiments were made. Alternatively sulphur trioxide may be less toxic than the dioxide in accordance with Swain's conclusion.

The experiments have confirmed the opinion of previous workers such as Swain that plants will endure much higher concentrations of SO_2 in relatively dry than they will in a humid atmosphere. Swain in fact records that a special observer was posted at a certain ore roasting factory to give notice when the meteorological conditions were particularly favourable for the action of SO_2 so that work could be stopped during these periods in order to reduce the damage done to the surrounding vegetation. Setterstrom & Zimmerman (1939) have also demonstrated the importance of low humidity and temperature in rendering plants less susceptible to SO_2 damage.

The conclusions, reached by careful experimentation, are in full agreement with the long practical experience of Kew gardeners, who, for many years have taken measures to reduce the humidity and lower the temperature in the greenhouses whenever the weather is foggy. Setterstrom & Zimmerman also found plants to be more resistant if grown in a good soil, which is likewise in accordance with horticultural experience.

In view of the toxicity of SO_2, it is interesting that Setterstrom & Zimmerman were able to show that if *Medicago sativa* is supplied with nutrients containing insufficient sulphur, the plant is able to absorb this element from the atmosphere with beneficial results when present in sufficiently low concentration.

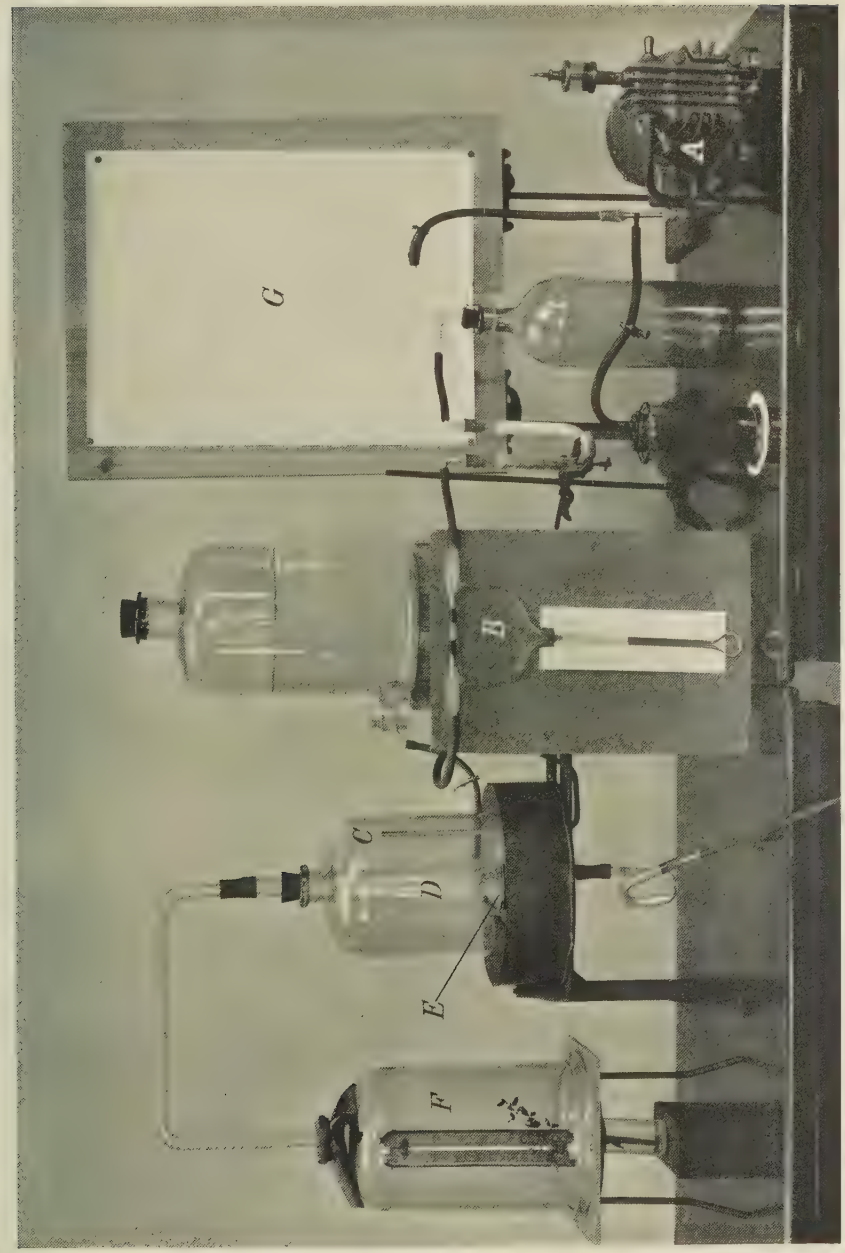
Setterstrom & Zimmerman found the concentrations of SO_2 in a greenhouse with the ventilators partly open to be about 10% less than its value outside the house and about 40% less when they were closed. Some of the readings in Table 1 above, particularly those obtained on 21 January 1941, confirm that the atmosphere in a greenhouse is less polluted than outside, but it seems that there is sometimes a greater difference than that recorded by Setterstrom & Zimmerman. There may quite well be variations of this kind in different localities and greenhouses. It is also important to note that Setterstrom & Zimmerman used quite different methods from those employed in the present investigation to estimate the SO_2 content of the air.

It is suggested that a paper covering is of value as a protection against SO_2, because of its ability to absorb this gas from the atmosphere. The work of Jarrell *et al.* (1936) on the deterioration of paper seems to support this view.

It is interesting that so many of the conclusions which can be drawn from the experimental results agree so closely with the empirical opinions of gardeners based on prolonged practical experience.

Summary

The shedding of flowers, buds and leaves of begonias and other plants, and the premature death of buds and flowers of orchids such as the calanthes are described. These and other symptoms, caused by town fog, are unfamiliar in country districts even during misty periods. These symptoms can be induced artificially by treating the plants with low concentrations of SO_2, comparable with those which occur in the atmosphere during foggy weather. These low concentrations of SO_2 are more toxic when the temperature and humidity are relatively high. A method is described by which the concentration of SO_2 in the atmosphere on different days or at different times during a single day may be compared. Tests by this method demonstrate that damage in the greenhouses is roughly proportional to the concentration of atmospheric SO_2. A method which proved to be very effective for removing SO_2 from the air on a laboratory scale, by filtering it through *Sphagnum* soaked in sodium carbonate solution, is described. Attempts were made to use this system on a larger scale in greenhouses, but further experiments are desirable before it can be recommended for general use. The behaviour of susceptible plants when grown under darkened bell jars or exposed to supplementary artificial illumination confirmed that the unhealthy symptoms described are caused by toxic substances in the atmosphere rather than the poor illumination which exists during foggy weather. At the same time it was also shown that if the plants were subjected for 12 hr. per day to light of reasonable intensity from neon tubes alone, or from a combination of neon tubes and mercury vapour lamps, earlier and more prolonged flowering were induced in begonias of the 'Gloire de Lorraine' type. There was some



METCALFE—Damage to greenhouse plants caused by town fogs with special reference to sulphur dioxide and light (pp. 301–15)

evidence that the general vigour of the plants as well as the size of the flowers were increased as well. Earlier flowering followed by vigorous vegetative growth were induced in *Euphorbia fulgens* by the same treatment, and earlier flowering in cinerarias and hyacinths. Various remedies for reducing the damage which occurs in foggy weather are suggested, ranging from simple precautions which can be applied by an intelligent gardener, to relatively complex arrangements for introducing purified air into the greenhouses. Chemical treatments intended to neutralize the SO_2 in the atmosphere were unsatisfactory.

I am particularly indebted to Sir Arthur W. Hill for his interest and encouragement throughout these investigations, as well as for arranging the necessary assistance from other individuals and institutions. Thanks are also due to Mr B. A. Ellis and Mr R. A. Jones of the Government Laboratory for their valuable help with the chemical aspects of the investigation. It would have been impossible to carry out the work without the co-operation of engineers from H.M. Office of Works, and from the gardens staff at Kew. The assistance of Mr C. C. Patterson and other members of the staff of the General Electric Co., Ltd., in designing and making one of the plant irradiators is much appreciated. Thanks are due to Prof. V. H. Blackman for his advice concerning the use of artificial light and for the loan of a lumeter, and to Mr W. G. Templeman for suggestions concerning the preparation of the manuscript. Plate 12 was prepared by Mr G. Atkinson.

REFERENCES

ARTHUR, J. M. & HARVILL, E. K. (1937). Plant growth from continuous illumination from sodium vapour lamps supplemented by mercury arc lamps. *Contr. Boyce Thompson Inst.* **8**, 438.

Bull. Mo. Bot. Gdn (1941), **29**, 4.

Committee on Trail Smelter Smoke of the National Research Council of Canada (1939). *Effect of Sulphur Dioxide on Vegetation.*

FALCONAR, A. (1931). Fog in relation to glasshouses. *Gdnrs' Chron.* ser. 3, **89**, 84.

JARRELL, T. O., HAWKINS, J. M. & VEITCH, F. P. (1936). Deterioration of book and record papers. *Tech. Bull. U.S. Dep. Agric.* no. 541. (See *Nature, Lond.* (1937), **139**, 890.)

JONES, H. TREFOR (1940). Agriculture and smoke pollution. *Smokeless Air*, Summer number.

METCALFE, C. R. (1937). The effect of atmospheric pollution on vegetation. *J. Park Administration*, **2**, 51–5.

OLIVER, F. W. (1891). On the effect of urban fog upon cultivated plants. *J.R. hort. Soc.* **13**, 139.

—— (1894). *J.R. hort. Soc.* **16**, 1.

RODENBURG, J. W. M. (1930). Kunstlichtkultuur. English Summary. *Meded. LandbHoogesch. Wageningen*, **34**, no. 8.

—— (1932). Kunstlichtkultuur II. German Summary. *Meded. LandbHoogesch. Wageningen*, **36**, no. 2.

—— (1936). Strawberries and neon light. *J.R. hort. Soc.* **61**, 504.

—— (1937). Der Einfluss der Tageslange im Zusammenhang mit der kunstlichen Pflanzenbeleuchtung im Winter. *Ber. dtsch. bot. Gesell.* **55**, 5–32.

SETTERSTROM, C. (1939). Sulphur dioxide content of air at Boyce Thompson Instit. *Contr. Boyce Thompson Inst.* **10**, 183–7.

SETTERSTROM, C. & ZIMMERMAN, P. W. (1939). Factors influencing susceptibility of plants to sulphur dioxide injury. I. *Contr. Boyce Thompson Inst.* **10**, 155–81.

EXPLANATION OF PLATE 12

Apparatus devised to treat plants and cut shoots with known low concentrations of SO_2 comparable with those which occur in the atmosphere during a fog. (For description see p. 306.)

(*Received* 28 *February* 1941)

VARIETAL SUSCEPTIBILITY OF PEAS TO MARSH SPOT

By H. H. GLASSCOCK

South-Eastern Agricultural College, Wye, Kent

The cause of marsh spot of peas (*Pisum sativum*) has not been satisfactorily explained although there is evidence (Löhnis, 1936; Pethybridge, 1936; Koopman, 1937; Ovinge, 1938; Heintze, 1938; De Bruijn, 1939; Lewis, 1939) that deficiency of available manganese in the soil may be at least contributory.

In the spring and summer of 1933 and 1934 a field survey was made of the incidence of marsh spot in crops of peas grown in Kent under contract for seedsmen, and correlation between the occurrence of the disease and certain soil types was found (Furneaux & Glasscock, 1936). In conjunction with this survey and with experimental field plots, other aspects of the disease, including varietal susceptibility, were studied.

Plots to test varietal susceptibility were laid out at Wye. Sowing was made without reference to earliness or lateness of the varieties because the names were not supplied by seedsmen until a later date. Each row was from a sample of that grown commercially in a field included in the survey. In 1933, fifty-five samples were sown, each row being divided into three parts (see Table 1).* In 1934, seventy-five samples were sown, each row being divided into four parts (see Table 1*a*).* In these tables, which show the relative position of the rows, B, C, E, H, X in the first column represent different firms of seedsmen and distinguish the source from which the seed was obtained; 2 before the letter denotes samples encountered during the second season; the variety name is given in the next column followed by the percentage of marsh spot appearing in each row-section. The last column gives the amount of marsh spot in the seed from which the crop was grown.

The field at Wye was selected primarily for uniformity of soil, and it was not known that marsh spot would appear in the crop. Of the samples of seed sown in 1933 only six gave crops which were completely free, the remainder being affected variously from 1 to 52%. Similarly, in 1934, no marsh spot was found in the crops from twenty-two samples, the remainder being affected in various degrees from 1 to 36%. Tables 1 and 1*a* show the incidence of marsh spot to be in relation to the rows irrespective of their position in the plot, demonstrating that no particular parts of the plot favoured the occurrence of the disease; e.g. in Table 1*a*, 'Onward' (2C53) was on average 20% affected, with a row on either side free from marsh spot, while 'Superb' (2C497), with an absence of marsh spot, had a row of 'Onward' on either side with over 20% marsh spot. In drawing conclusions from varietal data, one must take into account the significance of commercial 'varieties' at present in cultivation. Owing to the manner in which they are raised, such varieties are often only a mixture of heterozygous forms. Seedsmen commonly select 'strains' from these varieties and send them out under the original or their own descriptive names, so that the 'strain' of a given variety of one seedsman may differ genetically from that of

* Each part of the row received a different treatment, but these treatments proved to have no significant effect on the incidence of marsh spot, and in Tables 2–4*a* the percentage of marsh spot for any given row is the average of the percentages in all its sections.

TABLE 1. *Plot trials*, 1933

Wye ref. no.	Variety	% marsh spot: Plots			% marsh spot: Stock seed
B2	Bedford Champion	10	2	12	0
B4	Peerless	52	52	42	4
B6	Sensation	6	0	6	8
B7	Masterpiece	1	0	1	2
B10	Standards	1	0	1	1
B11	White-Seeded Dwarf	2	0	4	10
B12	Senator	3	0	5	55
C8	Aviator	4	2	1	0
C20	Gradus	32	15	6	?
C51	Alderman	6	26	9	3
C53	?	2	3	0	8
C73	Thomas Laxton	2	4	2	0
C85	Daisy	3	16	12	0
C104	Gradus	43	33	13	0
C108	Commonwealth	5	29	21	79
C151	Captain Cuttle	0	0	0	0
C170	Senator	0	1	3	0
C172	Lincoln	0	0	1	0
C209	Gradus	21	44	10	1
C355	Alderman	2	24	3	39
C367	Pioneer	16	3	11	0
C376	English Wonder	0	0	0	0
C418	Witham Wonder	0	0	2	10
C444	Alderman	6	19	7	29
C489	Progress	6	5	3	0
E1	?	1	2	2	5
E2	?	0	1	0	6
E3	?	3	3	15	0
E4	?	3	1	0	0
E5	?	2	3	2	2
E6	?	0	0	0	38
E7	?	7	10	13	0
E8	?	1	12	0	11
E90	?	2	4	7	1
E100	?	2	6	10	4
H1	Admiral Beatty	2	0	9	27
H2	Pilot	1	2	0	0
H3	Edward VII	0	2	2	0
H4	Duplex	1	0	0	1
H5	Marvellous	0	0	0	11
H6	Onward	10	15	10	1
H7	Edward VII	1	0	0	0
H8	Early Bird	1	0	1	1
H9	Thomas Laxton	3	3	0	0
H10	Progress	4	4	1	4
H11	Fenland Wonder	0	0	0	0
H12	Marvellous	1	3	1	13
H13	Petit Provençal	0	0	1	0
H14	Daisy	0	1	2	4
H15	Progress	5	3	0	7
H16	Onward	9	5	8	42
H17	Lincoln	0	1	0	1
X1	?	9	10	2	33
X2	?	4	1	4	4
X3	?	0	0	0	0

TABLE 1*a*. *Plot trials*, 1934

Wye ref. no.	Variety	% marsh spot: Plots				Stock seed
2C5	Aviator	3	1	0	2	1
2C8	Aviator	0	0	1	0	0
2C9	Gladstone	7	8	2	4	0
2C19	Gladiator	0	0	0	0	0
2C22	Superb	0	0	0	0	?
2C53	Onward	24	12	24	21	19
2C61	Meteor	0	0	0	0	3
2C62	British Lion	1	0	0	2	0
2C72	Onward	32	21	26	23	1
2C85	Daisy	13	17	17	17	1
2C88	Supreme	31	31	33	23	38
2C114	Harrison's Glory	9	9	5	13	15
2C116	Giant Stride	27	23	25	16	?
2C132	Aviator	0	0	0	0	1
2C140	Gladiator	0	0	0	0	0
2C161	Pilot	11	10	5	18	0
2C170	Senator	1	7	1	0	0
2C213	Little Marvel	1	1	3	1	1
2C310	Superb	0	0	0	0	0
2C337	Hundredfold	5	13	4	1	1
2C341	Superb	1	0	0	0	2
2C367	Pioneer	0	0	1	0	0
2C386	Pioneer	1	0	0	0	0
2C391	Blue Bird	1	0	0	0	1
2C410	Hundredfold	12	5	7	1	0
2C479	Witham Wonder	0	0	0	0	0
2C486	Thomas Laxton	2	5	1	1	4
2C489	Progress	11	9	16	27	0
2C493	Senator	1	1	2	4	0
2C495	Onward	20	26	25	20	8
2C497	Superb	0	0	0	0	0
2H1	Onward	25	26	20	12	28
2H2	Little Marvel	6	2	0	1	7
2H3	Thomas Laxton	2	4	1	2	0
2H4	Progress	29	9	8	14	88
2H5	Onward	23	12	21	24	10
2H6	Acquisition	35	34	36	33	13
2H7	Onward	22	11	22	37	0
2H8	British Lion	6	0	2	1	1
2H9	Duplex	13	9	13	29	0
2H10	Daisy	0	0	0	0	16
2H11	Meteor	0	0	0	0	4
2H12	Kelvedon Wonder	0	0	0	0	66
2H13	Kelvedon Wonder	0	0	0	0	58
2H14	American Wonder	0	0	0	0	0
2H15	English Wonder	0	0	0	1	0
2H16	Marvellous	0	0	0	0	13
2H17	Witham Wonder	0	0	0	0	0
2H18	Banqueter	0	1	0	0	0
2H19	Pride of the Market	0	0	0	0	0
2H20	Gradus	2	3	6	1	0
2H21	?	0	0	0	0	0
2H22	Dwarf Defiance	0	0	2	2	0
2H23	Kelvedon Wonder	0	0	0	0	0
2H24	Union Jack	0	0	0	0	0
2H25	Banqueter	0	0	0	0	0
2H26	Marvellous	0	1	1	0	2
2H27	Union Jack	0	0	0	0	0
2H28	Laxton's Progress	4	1	4	4	37

TABLE 1 *a* (*continued*)

Wye ref. no.	Variety	% marsh spot: Plots				Stock seed
2H29	Superb	0	0	1	0	0
2H30	Electricity	3	3	0	2	0
2H31	Blue Bird	0	0	3	0	0
2H32	Admiral Beatty	16	7	13	16	0
2H33	Bountiful	0	0	0	0	4
2H34	Alderman	5	0	6	7	2
2H35	Onward	14	5	6	11	2
2H36	?	1	1	0	0	2
2H37	Gradus	2	0	1	3	0
2B1	Pioneer	3	1	0	1	44
2B2	?	1	0	0	0	46
2B3	Senator	0	1	0	1	0
2B5	Onward	0	4	0	7	0
2B6	Evesham No. 1	1	0	0	0	0

another. In 1933, variety names were known of forty-one of the fifty-five samples, and these comprised twenty-eight different names. In 1934, variety names were known of seventy of the seventy-three samples, and these comprised thirty-seven different names. Only twelve of the varieties which had been sown in 1933 were, however, repeated the following year, so that, in all, a total of fifty-three varieties was tested in two seasons. In each season more than one row of some varieties was grown. These are grouped in Table 2 to facilitate comparison between the amount of marsh spot appearing in different rows of the same 'variety'.

Considering each season separately, the amount of marsh spot appearing in different rows of the same variety was usually of the same order of magnitude. In the three cases in which large differences occurred, they were from seeds of different sources. Of the eight rows of the variety 'Onward' grown in 1934, three were from seedsmen C, four from H and one from B. The samples from C and H, with one exception, show a remarkable uniformity in percentage of marsh spot. In both seasons more marsh spot was produced in 'Daisy' C than in 'Daisy' H. Because samples with the same variety name from stocks of different seedsmen often have somewhat different characteristics, it was necessary to consider the samples of a so-called 'variety' from each seedsman as a separate variety or 'strain' when comparing the influence of variety on the incidence of marsh spot. Viewed in this way, only twelve 'strains' were available for comparison (Table 3). None of these rows was severely affected in either year, and the range is probably too small to give adequate allowance for experimental error. Nevertheless, with the exception of 'Pioneer' there appears to be some relation between the sequences. 'Daisy' (C), 'Onward' (H), 'Progress' (C) and 'Progress' (H) are at the upper part of the list in both years. They also produced a higher percentage of marsh spot in 1934 than in 1933, while 'Senator' (B), 'Marvellous' (H) and 'Senator' (C) at the bottom of the list produced a lower percentage. A similar increase in range was also noted in the field samples suggesting that the seasonal factor does not necessarily affect susceptibility to the disease of all varieties in the same way.

The range of amounts of marsh spot in varieties on the trial grounds at Wye shows that conditions there were not as favourable to marsh spot as on many soils in Romney Marsh and East Anglia. Therefore, although the trials are a guide to relative susceptibility of a number of varieties, it cannot be said that any particular varieties are immune from the trouble or to what extent resistance plays a part. Trials for several years on a soil known to

TABLE 2. *Comparison between amounts of marsh spot encountered in different rows of the same varieties*

1933			1934		
Wye ref. no.	Variety	% marsh spot	Wye ref. no.	Variety	% marsh spot
C104	Gradus	29·6	2H20	Gradus	3·0
C209	,,	25·0			
C20	,,	17·6			
C51	Alderman	13·6	2H34	Alderman	4·5
C444	,,	10·6			
C355	,,	9·6			
H6	Onward	11·6	2C72	Onward	23·5
H16	,,	7·3	2H7	,,	23·0
			2C495	,,	22·8
			2C53	,,	20·3
			2H1	,,	20·3
			2H5	,,	20·0
			2H35	,,	9·0
			2B5	,,	2·8
C85	Daisy	10·3	2C85	Daisy	16·0
H14	,,	1·0	2H10	,,	2·5
C489	Progress	4·6	2C489	Progress	15·8
H10	,,	3·0	2H4	,,	15·0
H15	,,	2·6			
B12	Senator	2·6	2C170	Senator	2·3
C170	,,	1·3	2C493	,,	2·0
			2B3	,,	0·5
C73	Thomas Laxton	2·6	2C486	Thomas Laxton	2·3
H9	,,	2·0	2H3	,,	2·3
H12	Marvellous	1·6	2H26	Marvellous	0·5
H5	,,	0·0	2H16	,,	0·0
H3	Edward VII	1·3			
H7	,,	0·3			
C172	Lincoln	0·3			
H17	,,	0·3			
			2C410	Hundredfold	6·3
			2C337	,,	5·7
			2H8	British Lion	2·3
			2C62	,,	0·8
C8	Aviator	2·3	2C5	Aviator	1·5
			2C8	,,	0·3
			2C132	,,	0·3
C367	Pioneer	13·3	2B1	Pioneer	1·3
			2C367	,,	0·3
			2C386	,,	0·3
			2H31	Blue Bird	0·8
			2C391	,,	0·3
			2H21	,,	0·0
			2C341	Superb	0·3
			2H29	,,	0·3
			2C22	,,	0·0
			2C310	,,	0·0
			2C497	,,	0·0
			2H18	Banqueter	0·3
			2H25	,,	0·0
			2H24	Union Jack	0·0
			2H27	,,	0·0
			2C19	Gladiator	0·0
			2C140	,,	0·0
			2C61	Meteor	0·0
			2H11	,,	0·0
			2H12	Kelvedon Wonder	0·0
			2H13	,,	0·0

induce the disease severely were not possible in the present work, but a few firms of seedsmen have ascertained yearly the percentages of marsh spot occurring in crops of peas delivered to them by their growers. Although there are strong indications that variable factors such as soil and season influence the occurrence of the disease, it was considered that if the average percentage of marsh spot for a great number of crops of each variety was ascertained, then these varieties might fall into a sequence similar to that which would be expected if all were grown under identical conditions. A list of such averages for the seasons 1926–33 was obtained from a large firm which yearly places out contracts to farmers in a district very prone to produce marsh spot. The varieties grown in the trials at Wye and the names of which also appear on the seedsman's list are arranged for comparison in Tables 4 and 4*a* in descending order according to the amounts of marsh spot encountered. To facilitate comparison, the varieties are arbitrarily divided into groups, an asterisk being placed against those varieties which are not repeated in both columns of a group.

Table 4 shows that the corresponding sections of each column comprise the same varieties in a slightly different order. In Table 4*a* there is also a close relation between the

TABLE 3. *Comparison between amounts of marsh spot appearing in the same 'strains' of peas grown in the plot trials at Wye in* 1933 *and* 1934

1933			1934		
Variety (strain)	No. rows	Av. % marsh spot	Variety (strain)	No. rows	Av. % marsh spot
Pioneer (C)	1	13·3	Onward (H)	4	18·0
Daisy (C)	1	10·3	Daisy (C)	1	16·0
Onward (H)	2	9·5	Progress (C)	1	15·8
Progress (C)	1	4·6	Progress (H)	1	15·0
Progress (H)	2	2·8	Daisy (H)	1	2·5
Thomas Laxton (C)	1	2·6	Thomas Laxton (C)	1	2·3
Senator (B)	1	2·6	Thomas Laxton (H)	1	2·3
Aviator (C)	1	2·3	Aviator (C)	3	0·7
Thomas Laxton (H)	1	2·0	Senator (B)	1	0·5
Senator (C)	1	1·3	Pioneer (C)	2	0·3
Daisy (H)	1	1·0	Marvellous (H)	2	0·2
Marvellous (H)	2	0·8	Senator (C)	2	0·1

sequence of varieties. Thus the seedsman's list (Table 5) may be considered as a rough guide to the relative susceptibility of a number of varieties. Varietal descriptions by the various firms of seedsmen are often divergent in some respects, but three of the main characters commonly given are shape and size of seed and length of growing period. The data in Table 5 were compiled by one firm and the varietal characters are therefore most likely to be described with the same standards of reference. The average percentages of marsh spot as determined by the firm are given in descending order. The most severely affected varieties heading the list all have large, wrinkled seeds, are described as 'main crop', and are usually late maturing. At the other end of the list, the varieties have, with few exceptions, small or medium-sized seeds. Round-seeded varieties occur mainly in the latter half of the list. It may be deduced that large-seeded varieties tend to have long growing periods and that round seeds are usually small or medium in size and are produced by early varieties. Both 'Eldorado' and 'Talisman', the two most severely affected round-seeded varieties, have large seeds. The data in Table 5 do not show whether, if the same varieties were grown for several years in plots under uniform conditions, large size of seed or lateness of maturity alone would be the more important criterion of susceptibility.

No previous plot trials appear to have been carried out to ascertain the relative susceptibility of the common varieties. Quanjer (1915), however, stated that several kinds were attacked and these included grey and green peas. De Bruijn (1933) lists varieties noted in the report of the Dutch Seed Testing Station as being affected by marsh spot. The list also mentions Brown Beans and French Runner Beans. Ovinge (1935), from data collected

TABLE 4. *Comparison between apparent sequences of varietal susceptibility in peas from plots and from commercial crops*

Plot trials 1933			Seedsman's data		
Variety	No. rows	Av. % marsh spot	Variety	No. crops	Av. % marsh spot
Peerless	1	48·7	Peerless	16	81·0
Gradus	3	24·7	Onward	15	80·0
Pioneer	1	13·3	Daisy	26	65·3
Alderman	3	11·3	Gradus	58	58·7
Onward	2	9·5	Admiral Beatty	40	52·5
Bedford Champion	1	8·0	Bedford Champion	25	48·0
Daisy	2	5·7	Alderman	27	44·4
Admiral Beatty	1	3·6	Pioneer	47	40·4
Thomas Laxton	2	2·3	Lincoln	38	26·3
Senator	2	2·0	Early Bird	12	25·0
Marvellous	2	0·8	Marvellous	24	20·8
Witham Wonder	1	0·6	Thomas Laxton	93	16·1
Early Bird	1	0·6	Senator	45	13·3
Lincoln	2	0·3	Witham Wonder	34	8·8
English Wonder	1	0·0	English Wonder	42	7·1

TABLE 4*a*

Plot trials 1934			Seedsman's data		
Variety	No. rows	Av. % marsh spot	Variety	No. crops	Av. % marsh spot
Giant Stride	1	22·8	Onward	15	80·0
Onward	8	16·0	Giant Stride	10	70·0
Admiral Beatty	1	13·0	Dwarf Defiance*	33	66·6
Daisy	2	9·3	Daisy	26	65·3
Gladstone	1	5·3	Laxton's Progress	40	60·0
Alderman	1	4·5	Gradus	58	58·7
Laxton's Progress	1	3·3	Admiral Beatty	40	52·8
Gradus	1	3·0	Gladstone	14	50·0
Thomas Laxton*	2	2·3	Alderman	27	44·4
Little Marvel	2	1·9	Pioneer	47	40·4
Senator*	3	1·6	Little Marvel	56	26·7
British Lion	2	1·5	Kelvedon Wonder*	17	23·5
Dwarf Defiance*	1	1·0	Marvellous*	24	20·8
Pioneer	3	0·6	British Lion	35	17·1
Blue Bird	3	0·4	Thomas Laxton*	93	16·1
English Wonder	1	0·3	Blue Bird	43	13·9
Marvellous*	2	0·3	Senator*	45	13·3
Witham Wonder	2	0·0	Bountiful	41	12·2
Kelvedon Wonder*	3	0·0	Witham Wonder	34	8·8
American Wonder	1	0·0	English Wonder	42	7·1
Union Jack	2	0·0	American Wonder	24	4·1
Bountiful	1	0·0	Union Jack	12	0·0

on commercial crops, concluded that there was an appreciable difference in the occurrence of the disease in different varieties. Lacey (1934) observed that the disease was worse in the late or main crop varieties grown in her trials than in the early kinds.

In connexion with size of seed, de Bruijn (1933) noted that most disease was to be found amongst the heaviest peas of a sample, particularly in those samples which were less badly

TABLE 5. *Average amounts of marsh spot produced in numerous commercial crops of peas grown in East Anglia in the years 1926–33. From data supplied by seedsman*

% marsh spot	Variety	Season	Size of seed	Round (R) or wrinkled (W)
81	Peerless	M/C, late	Large	W
80·9	Prestige	Late	Large	W
80	Sutton's V.C.	M/C, late	Large	W
80	Onward	M/C	Large	W
70	Giant Stride	M/C, late	Large	W
66·6	Dwarf Defiance	M/C	Large	W
66·6	Glory of Devon	Late	Large	W
65·3	Daisy	M/C	Large	W
63·3	Sharpe's Standard	M/C, late	Large	W
61·5	Royal Salute	M/C	Large	W
60	Laxton's Progress	Early	Large	W
58·7	Gradus	Early	Medium	W
57·9	Eldorado	Early	Large	R
57·8	Sharpe's Liberty	Late	?	W
53	Veitche's Perfection	Late	Large	W
53	Sutton's Hundredfold	Early	Large	W
52·5	Admiral Beatty	M/C	Large	W
50	Sharpe's Queen	Late	Large	W
50	Gladstone	Late	Medium	W
50	Renown	M/C	Medium	W
48	Bedford Champion	Early	Medium	W
47·4	Talisman	Early	Large	R
46·4	Sherwood	M/C, early	Medium	W
45·4	Sutton's Excelsior	2nd early	Small	W
44·4	Alderman	M/C, late	Large	W
40·4	Pioneer	2nd early	Medium	W
38·4	King Edward	2nd early	Large	W
36·8	Lancashire Lad	2nd early	Large	W
36·8	Sharpe's Presidẽnt	M/C	Medium	W
36·3	Phenomenon	M/C	Large	W
36·3	Telephone	M/C	Medium	W
34·6	Benefactor	Early	Medium	R
33	Manchester Man	M/C	Large	W
26·7	Little Marvel	Early	Small	W
26·5	Sharpe's Meteor	Early	Small	R
26·3	Lincoln	M/C	Small	W
25	Early Bird	Early	Medium	R
23·5	Kelvedon Wonder	Early	Small	W
21	Telegraph Super	M/C	Medium	R
20·8	Marvellous	2nd early	Medium	W
20	Quite Content	M/C	Medium	W
17·1	British Lion	Early	Large	R
16·1	Thomas Laxton	Early	Medium	W
15·7	William Hurst	Early	Small	W
14·7	Primo	Early	Large	R
13·9	Blue Bird	Early	Medium	W
13·6	World's Record	Early	Small	W
13·3	Senator	M/C	Medium	W
12·5	Tip Top	Early	Small	R
12·2	Bountiful	Early	Medium	R
11·7	Ne Plus Ultra	Late	Large	W
10	Autocrat	Late	Large	W
8·8	Witham Wonder	2nd early	Small	W
7·7	Essex Star	M/C	Medium	R
7·1	English Wonder	Early	Small	W
6·7	Sharpe's Unique	Early	Small	R
5·9	Laxton's Superb	2nd early	Large	R
4·1	American Wonder	Early	Small	W
0·0	Union Jack	M/C	Large	W
0·0	Earliest of All	1st early	Small	R
0·0	Early White Seedling	1st early	Small	R
0·0	First and Best	1st early	Small	R
0·0	King of Serpette	M/C	Small	R
0·0	Serpette	M/C	Small	R
0·0	William the Conqueror	2nd early	Small	R

affected, and this point was confirmed by Furneaux & Glasscock (1936). In addition, from trials with different fertilizers, de Bruijn concluded that a protracted ripening period encouraged the development of marsh spot while the trouble was less in the seeds of those plants in which maturation had been hastened. The varietal characteristics of large size of seed and late season, which are seen from the above lists to favour the incidence of the disease seem, therefore, to influence its occurrence also when found within a given variety.

Summary

Plot trials at Wye in 1933 and 1934 demonstrated that varieties differ in their susceptibility to the disease and numerous varieties are arranged in tables to show their relative susceptibility. The amount of marsh spot found in commercial crops of seed of numerous varieties of peas grown in East Anglia during a period of eight years was supplied by a large firm of seedsmen. The relative susceptibility of these varieties approximates to that found in the plot trials at Wye for varieties of the same name. Using the percentages of marsh spot and the varietal characteristics given by the firm, it is shown that varieties which are late maturing and have large seeds were more severely affected than early maturing varieties with small seeds. Roundness of seed was also associated with earliness of maturity and resistance to disease.

REFERENCES

De Bruijn, H. L. G. (1933). Kwade harten van de erwten. *Tijdschr. PlZiekt.* **39**, 281.
—— (1939). Mangaangebrek, oorzaak van de kwade harten van erwten. *Tijdschr. PlZiekt.* **45**, 106.
Furneaux, B. S. & Glasscock, H. H. (1936). Soils in relation to marsh spot of pea seed. *J. agric. Sci.* **26**, 59.
Heintze, S. G. (1938). Readily soluble manganese of soils and marsh spot of peas. *J. agric. Sci.* **28**, 175.
Koopman, C. (1937). Invloed van mangaansulfaatbespruiting tegen kwaadhartigheid bij Schokkerwten. *Tijdschr. PlZiekt.* **43**, 64.
Lacey, M. S. (1934). Studies in bacteriosis. XXI. An investigation of marsh spot of peas. *Ann. appl. Biol.* **21**, 621.
Lewis, A. H. (1939). Manganese deficiencies in crops. I. Spraying pea crops with solutions of manganese salts to eliminate marsh spot. *Emp. J. exp. Agric.* **7**, 150.
Löhnis, M. P. (1936). Wat veroorzaakt kwade harten in erwten? *Tijdschr. PlZiekt.* **42**, 159.
Ovinge, A. (1935). Het optreden van kwade harten in schokkers in Zeeland in 1934. *Landbouwk. Tijdschr.* **47**, 375.
—— (1937). Kwade harten in schokkers. *Tijdschr. PlZiekt.* **43**, 67.
—— (1938). Kwade harten-proeven in Zeeland in 1937. *Tijdschr. PlZiekt.* **44**, 208.
Pethybridge, G. H. (1936). Marsh spot in pea seeds: is it a deficiency disease? *J. Minist. Agric.* **43**, 55.
Quanjer, H. M. (1915). Kwade pitten in de erwten. *Zeeuwsch. Landbouwbl.* **7**, no. 250.

(*Received* 25 *February* 1941)

SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

VII. SURVIVAL OF *OPHIOBOLUS GRAMINIS* ON THE ROOTS OF DIFFERENT GRASSES

By S. D. GARRETT

Rothamsted Experimental Station, Harpenden, Herts

Occasional observations on the relative susceptibility of different grass species to infection by *Ophiobolus graminis* are to be found in the majority of papers on the take-all disease; extensive experimental tests have been conducted only by Kirby (1922, 1925), Padwick & Henry (1933), Russell (1934) and Winter (1939). The tests were carried out in glasshouse pots; observations were made by Kirby after 5 months in the first and after 1 year in the second test, by Padwick & Henry after $7\frac{1}{2}$ weeks, by Russell after 14 weeks and again after 7 months, and by Winter after $5\frac{1}{2}$ weeks and again after 5 months. All these authors supplemented their pot tests by observations on wild grasses collected from the field. On tabulating their results, and comparing them with a series of three similar tests made by the writer, fair agreement was observed except for the first trial by Kirby (1922). In this trial, Kirby obtained notably fewer positive results than the other investigators, recording as resistant to infection a number of species which were otherwise unanimously agreed to be susceptible. In his second trial, Kirby (1925) expressed his results solely by the relative abundance of perithecia on the inoculated plants after 1 year. This criterion was certainly consistent with Kirby's (1925) expressed view that 'the ascospores constitute the inoculum for the vast majority of infections', though this view has since been challenged (Garrett, 1939). Moreover, Müller-Kögler (1938) has since reported that numerous perithecia may be formed superficially in the tissues of plants resistant to the further progress of infection; this observation has been confirmed by the writer. With the exception of Kirby, therefore, these different investigators agreed that species of the following genera were susceptible to attack, viz. *Agropyron*, *Bromus*, *Festuca*, *Hordeum* and *Lolium*, and also the species *Dactylis glomerata*; as resistant were classified species of *Agrostis* and *Avena* and also the species *Phleum pratense*. No conclusion could be reached concerning the susceptibility of *Poa* spp., which appeared more variable in this respect than species of other genera.

Although infection by *Ophiobolus* may impair the efficiency of individual plants of susceptible grass species, even leading in extreme cases to death of the individual, it is rarely likely to be the factor limiting the productivity of the pasture as an ecological whole. Such loss of productivity can only occur when the dominant species is highly susceptible to attack by *Ophiobolus*; an example is furnished by the almost pure stands of *Hordeum murinum* seen in South Australia. The agricultural importance of grass hosts is to be sought firstly in the origin of the disease in virgin grasslands, and secondly in the indefinite survival of the fungus in temporary pastures from one cereal crop to the next, as well as

on susceptible grass weeds on fallows and in other crops. Neither in Canada nor in Australia is the appearance of take-all in the wheat crops generally long delayed after the plough has first been put into the virgin land. Thus Sanford (1929), after surveying the Alberta wheat crops in 1928 for root rots, chief amongst which was take-all, reported: 'Where wheat follows breaking, the 0·9 (per cent) loss class was not exceeded, and a few of the fields have no recorded loss. These observations correspond with the data from surveys made in 1927 and earlier. However, there is a marked increase in the severity of the disease on the second crop following breaking. The severity of foot-rot in the third and fourth successive crop of wheat following breaking is often very marked, and the distribution of the disease is usually fairly general.' From a similar survey of the cereal crops made in Saskatchewan, Russell (1934) concluded: '*O. graminis* is present in the virgin sod; it seldom damages the first crop of wheat on new land to any great extent, but the second and subsequent crops of wheat may be severely diseased unless the wheat crops are alternated with summer fallow or crops of highly resistant plants.' From South Australia, Griffiths (1933) remarked that take-all 'frequently appears after the first crop, particularly when the foolish policy of growing several wheat crops in succession is adopted. There is no doubt that much of the present trouble has been caused by the general practice in the past of over-cropping new mallee areas with wheat.' An English survey now in progress is showing that *Ophiobolus* is apparently widely distributed in the old grassland areas of this country.

These aspects of the take-all problem appeared to demand further study, in view of the lack of direct experimental evidence as to the relative efficiency of different species of grasses as propagators of *Ophiobolus*. Whilst agreement between the results of the different inoculation trials discussed above had been fairly good, there were some discrepancies. For instance, Winter found that 38 days after inoculation, plants of *Agrostis spica-venti* seemed outwardly healthy; the roots appeared scarcely infected, and runner hyphae were sparsely distributed, in agreement with other observations on the resistance to infection of *Agrostis* spp. Yet, after 5 months, all inoculated plants were dead, and bore perithecia. Again, in tests by the writer, certain species, such as *Anthoxanthum odoratum*, *Cynosurus cristatus* and *Poa* spp., showed a variable amount of root discoloration, and were difficult to classify definitely either as susceptible or as resistant. Even apparently resistant species such as *Avena elatior* or *Phleum pratense* showed occasional discoloured lesions on the roots. In general, infection of the seminal roots was more severe than that of the crown roots in all species examined. Little reliance could be placed on the presence or absence of runner hyphae on the roots as a criterion of fundamental susceptibility, since Müller-Kögler (1938) has shown that *Ophiobolus* hyphae will grow down the tap-roots of many dicotyledonous seedlings, which afterwards throw off the infection. Turner (1940) has demonstrated that the runner hyphae will grow along both seminal and crown roots of oats (albeit less rapidly than along those of wheat), but that infection proceeds no further after initial penetration of the outer root cortex. In these grass host investigations, runner hyphae were observed by Winter and by the writer on the roots both of *Avena elatior* and of *Phleum pratense*, which otherwise appeared to be resistant.

These difficulties were appreciated by Padwick (1935), who remarked 'the mere fact that a plant is susceptible to attack by wheat foot-rotting pathogenes under the unusual conditions of experimental inoculation, especially in sterilized soil, does not give an adequate indication of the role which it may play in the problem of foot-rot of wheat'. Padwick

therefore employed a direct technique for determining the power of different grass species to act as perpetuators of *Ophiobolus*. He sowed seed of four species of grasses, *Agropyron tenerum*, *A. cristatum*, *A. repens*, and *Bromus inermis*, in pots of black loam soil artificially inoculated with *Ophiobolus*; ten pots were sown with each species of grass, and two additional series of pots were included in the experiment, one left fallow and the other sown with *Neslia paniculata* (ball mustard), a dicotyledon. The plants were allowed to grow in the glasshouse for 1½ months, after which the tops were cut off, and twenty-five wheat seeds sown amongst the roots in each pot. After 3 weeks, the wheat seedlings were removed and the degree of infection recorded. No infection was observed on those seedlings in the fallow unsterilized soil, showing that the fungus had died out during the 1½ months' interval between inoculating the soil and planting the test wheat seedlings; in the fallow-sterilized soil a very light infection of the wheat seedlings was recorded. No infection occurred in either soil series planted with the ball mustard. Appreciable infection of the test wheat seedlings occurred after sowing with all four grasses, the maximum disease rating being one of 16%. In another type of experiment, Padwick demonstrated that *Ophiobolus* not only survived but also spread for distances of up to 12 in. in 7½ weeks, from a trench filled with corn-meal soil inoculum, along the roots of *Agropyron tenerum*, of *A. repens* and of wheat, respectively.

Padwick's technique was therefore adopted and extended for the present investigation; whereas Padwick only made one observation on the susceptibility of each grass species, the experiments here described provided for serial observations, through the planting of test wheat seedlings at regular intervals on the inverted sods of the inoculated grasses. No dicotyledons other than *Trifolium pratense* were included in these experiments, since there is no field evidence to suggest that species of plants outside the Gramineae can carry *Ophiobolus*. This conclusion has been confirmed by the studies of Müller-Kögler (1938), who investigated the reaction of seventy-three dicotyledons to inoculation with *Ophiobolus* under soil conditions extremely conducive to infection, and found none to be infected to any appreciable extent. He divided the species into four groups: (1) showing no signs of infection; (2) showing growth of runner hyphae along the exterior of the roots, but a minimum of internal infection; (3) showing rapid infection of the primary root cortex, which was sloughed off by formation of a pericyclic epidermis, with termination of the infection; (4) showing rapid infection of the primary root cortex but no further progress of infection, presumably owing to protoplasmic incompatibility between host and parasite.

Experimental

The general plan of these experiments was as follows. The seed of different species of grasses was planted in wooden boxes over the minimal amount of *Ophiobolus* inoculum necessary to secure uniform infection of susceptible species; after a 2-months period of growth, the grass tops were cut off and the sods inverted in the boxes, to simulate ploughing up. The boxes were stacked in a compartment of the glasshouse maintained as far as possible at a constant temperature; at intervals, test wheat seedlings were planted in the inverted sods of the different grasses, and examined for root infection after 3 weeks' growth. In this way, the degree of survival of *Ophiobolus* on the root system of the different species of grasses could be determined.

Exp. I. The following species were employed:

Agrostis alba var. *stolonifera*	*Festuca pratensis*
A. tenuis	*F. rubra*
Alopecurus pratensis	*F. rubra* var. *fallax*
Anthoxanthum odoratum	*Lolium italicum*
Avena elatior	*L. perenne*
Cynosurus cristatus	*Phleum pratense*
Dactylis glomerata	*Poa pratensis*
Festuca ovina	*P. trivialis*

The experiment was carried out in wooden seed boxes, $35 \times 22 \times 8$ cm., filled with a mixture of three parts sand to one part of Harpenden allotment soil (by volume). The soil was diluted with sand so as to provide optimum conditions for *Ophiobolus* to infect and spread along the roots of the grasses (Garrett, 1936). Seven boxes were planted with each grass species, and fourteen boxes were left fallow, to serve as a check on the occurrence of the fungus in the unsterilized allotment soil. Inoculation was effected by spreading 150 g. of a sand + 3% cornmeal culture of *Ophiobolus* (isolate obtained from wheat at Wareham, Dorset, in 1938) as a layer approximately 1·25 mm. deep on the surface of the soil in each flat before sowing the grass seed, which was then covered to a depth of approximately 5 mm. with the same soil mixture. The boxes were planted on 24 July 1939, and placed outside in groups of twenty inside wooden frames covered with a wide-mesh white window netting, to keep out sparrows. A standard dose of plant nutrient solution was given to every box at 4, 5 and 6 weeks after planting; the total addition of nitrogen corresponded approximately to a field dressing of 1 cwt. of nitrate of soda/acre.

At the end of 2 months the majority of grasses had made satisfactory growth. Establishment has been good in all boxes, though the two *Lolium* spp. had appeared somewhat sickly in the early stages of growth, probably as a result of infection. The only grass which seemed to be dying in patches in consequence of attack of *Ophiobolus* was *Alopecurus pratensis*; growth was poor in the boxes of *Festuca ovina*, *F. rubra* and *F. rubra* var. *fallax*, but, in the absence of uninoculated control boxes, the poor growth of the inoculated grasses could not be definitely ascribed to infection by *Ophiobolus*. At the end of the 2 months' growing period, an individual plant from each one of the seven boxes belonging to each species was lifted for examination under the binocular dissecting microscope. An attempt was made to separate the different species into resistant and susceptible groups, according to the degree of root discoloration (Table 1).

TABLE 1. *Results of root examination under binocular dissecting microscope*

Susceptible	Resistant
Alopecurus pratensis	2 *Agrostis* spp.
Dactylis glomerata	*Anthoxanthum odoratum*
4 *Festuca* spp.	*Avena elatior*
2 *Lolium* spp.	*Cynosurus cristatus*
Poa pratensis	*Phleum pratense*
	Poa trivialis

In all species, infection of the seminal roots, as estimated by visible discoloration, was greater than that of the crown roots. The results of this examination coincided with those of previous tests made by the writer, and also in a general way with those of Padwick & Henry (1933), Russell (1934) and Winter (1939).

On 26 and 28 Sept. the tops of the grasses were cut off with scissors close to soil level,

and the sods taken out of the boxes and replaced in an inverted position. The boxes were then stacked in covered piles in the glasshouse; the weekly mean air temperature varied from 11 to 19° C., with an average of 15·4° C., during the remainder of the experiment. A sample of two boxes from each grass species at 3 and 7 weeks, and of one box at 12, 17 and 22 weeks (double these numbers were available in the fallow series), was taken for planting with test wheat seedlings; fifty-five seeds of Little Joss wheat were planted per box, in order to obtain not less than fifty seedlings. After 3 weeks' growth in the glasshouse, the wheat seedlings were washed out and examined for root infection, which was expressed as number of seminal roots infected per seedling (out of a possible maximum of six which can be produced by the seedling). Examination was made by naked eye alone of the root system floating out in water over a white background; a root was held to be infected when it showed one or more lesions with characteristic discoloration of the vascular cylinder. The end-point of infection thus selected does not always coincide with the complete

TABLE 2. *Survival of* Ophiobolus graminis *on roots of grasses, estimated by mean number of infected seminal roots per plant in test wheat seedlings, planted at intervals after inversion of grass sods*

	After 3 weeks	After 7 weeks	After 12 weeks	After 17 weeks	After 22 weeks	Mean infection figure for the 5 samples
Agrostis tenuis	4·76	4·69	3·77	3·40	2·78	3·88
A. alba var. *stolonifera*	4·50	4·54	3·79	3·08	3·43	3·87
Poa pratensis	4·58	4·42	3·26	2·71	1·87	3·37
Festuca rubra var. *fallax*	4·33	4·25	3·66	2·04	2·38	3·33
F. ovina	4·36	4·21	2·78	2·25	1·28	2·98
Alopecurus pratensis	4·44	4·08	2·00	1·34	2·13	2·80
Lolium italicum	4·27	3·83	2·41	2·28	—	—
L. perenne	4·36	3·74	2·36	1·76	1·51	2·75
Dactylis glomerata	4·16	4·41	2·43	1·61	0·83	2·69
Cynosurus cristatus	3·32	4·57	2·69	1·67	0·59	2·57
Festuca rubra	3·25	3·37	3·48	1·83	—	—
F. pratensis	3·37	3·51	2·57	1·06	0·30	2·16
Anthoxanthum odoratum	3·55	3·04	1·13	0·53	0·18	1·69
Avena elatior	1·75	2·24	1·91	0·80	0·31	1·40
Poa trivialis	2·53	1·94	0·38	0·27	0·04	1·03
Phleum pratense	2·36	0·60	0·40	0·34	0·17	0·77
Fallow	0·19	0·03	0·02	0·07	0·04	0·07

absence of *Ophiobolus* from the root system of the test wheat seedling; thus a light infection by a few hyphae may not produce a discoloured lesion visible to the naked eye. This distinction, although an important one, in no way detracts from the value of the selected end-point for its present purpose, viz. estimation of the *relative* amounts of *Ophiobolus* inoculum in the different boxes. It was unfortunate that no additional distinction could be made for this purpose between heavily and lightly infected roots, except at the expense of too much labour; as it is, the results give only a conservative estimate of the differences actually observed in degree of infection of the test wheat seedlings. The results of this first experiment are given in Table 2; the grasses are arranged in descending order of effectiveness as propagators of *Ophiobolus*.

It is impossible to draw a line at any level through this tabulation of results, such that it separates propagators from non-propagators of *Ophiobolus*. There is undoubtedly, however, a great difference between the species at either end of the Table. The results confirm the

resistance of *Phleum pratense* already reported by Padwick & Henry (1933), Russell (1934) and Winter (1939); as was to be expected, *Avena elatior* is also nearly at the bottom of the table. It is also interesting to see that the distinction made between *Poa pratensis* and *P. trivialis* in Table 1 is confirmed in Table 2. One major discrepancy between the results of the preliminary examination and those of the final test of susceptibility requires explanation, viz. the behaviour of the two *Agrostis* spp. Both in this and in previous root examinations of inoculated *Agrostis* spp. under the binocular dissecting microscope, runner hyphae were relatively scarce and discoloration only light by comparison with species of *Lolium* and *Festuca*. A clue to this paradox is possibly to be found in Winter's results. At the 38-day examination of his inoculated plants, the roots of *Agrostis alba* var. *stolonifera* showed neither browning nor external mycelium, whilst those of *A. spica-venti* exhibited occasional discoloration and sparse mycelium. After 5 months, the roots of *A. alba* var. *stolonifera* showed light browning with sparse mycelium; all plants of *A. spica-venti*, however, were now dead, presumably through infection. Furthermore, Winter had observed *A. spica-venti* ('Windhalm') to be very susceptible to infection by *Ophiobolus* in the field, stating: 'Insbesondere gibt es aber zu denken, dass Ophiobolosenester in der Regel stärker mit Windhalm verunkrautet sind, und dass dieser stets Anzeichen schweren Ophiobolosebefalls erkennen lässt. Die Pflanzen sind schwächer entwickelt, ihre Wurzeln sind zerstört, die Halmbasis is geschwärzt, und in einzelnen Fällen treten Perithecien auf.'

Exp. II. The result of most practical interest from Exp. I was the relatively rapid disappearance of *Ophiobolus* after *Avena elatior* and *Phleum pratense*, since these species might sometimes be employed in place of the more susceptible *Lolium* spp. for temporary leys. The following series were compared in this experiment:

Lolium perenne	*Avena elatior*
L. italicum	*Trifolium pratense*
Phleum pratense (Scotch seed)	Fallow
P. pratense (Aberystwyth S. 51)	

The experiment was set up in the same manner as Exp. I, except that 120 g. of cornmeal-sand inoculum was deemed sufficient, giving a layer approximately 1 mm. in depth on the surface of the soil in each box. Another *Ophiobolus* isolate, obtained from wheat at Woburn in 1939, was used for inoculation. The boxes were inoculated and planted on 6 Sept. 1940, and this time were placed in a compartment of the glasshouse.

Approximately the same addition of nutrient solution was made to the boxes as in Exp. I. Establishment of plants was good in all boxes, but the stand was too thick in the boxes of broad red clover, which were therefore thinned out 3 weeks after planting. The growth of the tops was not as vigorous as in Exp. I, presumably owing to the poorer light conditions at this season of the year; some trouble was experienced with *Botrytis* sp. in some of the timothy boxes. After 2 months' growth, the tops of the grasses were cut off, and the sods inverted in the boxes, on 13 and 14 Nov. The root mat was by no means so strongly developed as in the boxes of Exp. I, corresponding with the poorer growth of tops. The boxes were stacked in piles in the same compartment of the glasshouse, the weekly mean air temperature of which varied from 15 to 19° C., with an average of 17·6° C., over the 4 months' sampling period. At monthly intervals from 1 to 4 months after inversion of the sods, a sample of three boxes was taken from each series; fifty-five Little Joss wheat seeds were sown per box. At 4 months, only the two *Lolium* series were sampled. The results are given in Table 3.

Comparing the results with those of Exp. I, the figures for *Phleum pratense* are seen to be closely comparable with those in Table 1. The initial infection after the two *Lolium* spp. is also of the same order as that obtained in Exp. I, but infection fell more rapidly at the latter samplings. This is probably to be attributed partly to the higher temperature of incubation of the stacked boxes and partly to the less vigorous original growth of the grasses in this experiment, leading to a smaller root mat, which was indeed observed to decompose more quickly than that obtained in Exp. I. *Avena elatior* again occupied a position intermediate between *Phleum pratense* and the two *Lolium* spp. It is interesting to note that the fungus tended to die out more rapidly under *Trifolium pratense* than in the fallow soil (cf. similar result obtained by Padwick (1935) with *Neslia paniculata*).

TABLE 3. *Survival of* Ophiobolus graminis *on roots of grasses, estimated by mean number of infected seminal roots per plant in test wheat seedlings, planted at intervals after inversion of grass sods*

	After 1 month	After 2 months	After 3 months	After 4 months
Lolium italicum	4·29	2·59	1·11	0·80
L. perenne	4·49	2·92	0·86	0·91
Phleum pratense (Scotch)	2·43	0·59	0·20	—
P. pratense (Aberystwyth S. 51)	2·89	0·58	0·14	—
Avena elatior	2·72	1·81	0·65	—
Trifolium pratense	0·07	0·05	0·04	—
Fallow	0·34	0·06	0·03	—

APPLICATION TO PRACTICE

The crop sequence—barley, rye-grass and clover ley, wheat—is not uncommon in English rotations; it occurs, for instance, in the traditional Norfolk four-course rotation. Since rye-grass can perpetuate *Ophiobolus*, this crop sequence must tend to perpetuate take-all, albeit at a low level, on many farms. Two courses are open to farmers on the light-textured chalk soils on which take-all is most troublesome. The first is to break up the seeds ley not later than June, and bastard fallow the land in preparation for wheat; a crop of mustard can be taken if the wheat bulb fly is considered likely to lay eggs on the fallow. The 4 months' period from breaking in June to drilling in October gives an interval in which most of the *Ophiobolus* will have died out from the infected rye-grass roots, if the fallow has been kept clean, or the growth of mustard a good one. The alternative is to replace the rye-grass component of the temporary ley by one or more of the comparatively resistant grasses, as already proposed elsewhere (Garrett, 1940). Mr William Davies, of the Welsh Plant Breeding Station at Aberystwyth, has suggested the following two seeds mixtures as suitable for special use where take-all has become a serious problem:

	Mixture 1 lb.	Mixture 2 lb.
Timothy (Scotch)	8	10
Timothy (S. 51)	4	5
Tall oat grass	6	—
Broad red clover	2	2
Late-flowering red clover	2	2
Late red clover (S. 123)	2	2
White clover (S. 100)	1	1

The value of rye-grass for temporary leys is too well established to warrant its replacement by either of these mixtures except under special circumstances. Cases have occurred,

however, notably on the Wiltshire Downs and on the Yorkshire Wolds, where yields of wheat have been so poor after rye-grass leys, whether on account of frit-fly, take-all or yet other causes, that farmers have resorted to leys of pure clover. Under such circumstances, therefore, these special seeds mixtures may find useful employment.

Summary

Sixteen species of grasses were inoculated with *Ophiobolus graminis*, and their roots examined under the binocular dissecting microscope for runner hyphae and discoloured disease lesions. Whilst some species were obviously susceptible and others showed few signs of root infection, there were yet other species which were difficult to classify either as susceptible or as resistant. The effectiveness of these 16 grasses as perpetuators of *Ophiobolus* was therefore directly tested, as follows. The seed was sown in contact with a minimal amount of inoculum in boxes of a light-textured soil; 2 months after planting, the grass tops were cut off, and the sods inverted in the boxes. The degree of survival of *Ophiobolus* in the inverted sods of the different grasses was determined at approximately monthly intervals by the planting of test wheat seedlings. Whilst all 16 species propagated *Ophiobolus* to some extent, as compared with a negligible survival in fallow soil and under clover, there were notable differences in the longevity of the fungus under different grasses. The resistance of *Phleum pratense*, reported by previous investigators, was confirmed, and seeds mixtures employing this grass and *Avena elatior* in place of *Lolium* spp. were suggested for use on heavily-infected land.

I have pleasure in thanking Mr William Davies for his interest in this investigation, and for suggesting the two seeds mixtures given above; my thanks are also due to Messrs Dunns Farm Seeds Ltd., of Salisbury, and to Messrs Gartons Ltd., of Warrington, for gifts of wheat and grass seed.

REFERENCES

Garrett, S. D. (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1939). Soil conditions and the take-all disease of wheat. IV. *Ann. appl. Biol.* **26**, 47.

—— (1940). Temporary leys and the take-all and whiteheads disease of wheat and barley. *J. Minist. Agric.* **47**, 134.

Griffiths, R. L. (1933). Take-all. Incidence and control on the lighter soils of the Mallee. *J. Dep. Agric. S. Aust.* **36**, 774.

Kirby, R. S. (1922). The take-all disease of cereals and grasses. *Phytopathology*, **12**, 66.

—— (1925). The take-all disease of cereals and grasses caused by *Ophiobolus cariceti* (B. & Br.) Sacc. *Mem. Cornell agric. Exp. Sta.* **88**.

Müller-Kögler, E. (1938). Untersuchungen über die Schwarzbeinigkeit des Getreides und den Wirtspflanzenkreis ihres Erregers (*Ophiobolus graminis* Sacc.). *Arb. biol. Abt.* (*Anst.—Reichsanst.*) *Berl.* **22**, 271.

Padwick, G. W. (1935). Influence of wild and cultivated plants on the multiplication, survival and spread of cereal foot-rotting fungi in the soil. *Canad. J. Res.* **12**, 575.

Padwick, G. W. & Henry, A. W. (1933). The relation of species of *Agropyron* and certain other grasses to the foot-rot problem of wheat in Alberta. *Canad. J. Res.* **8**, 349.

Russell, R. C. (1934). Studies of take-all and its causal organism, *Ophiobolus graminis* Sacc. *Bull. Dep. Agric. Can.* **170**.

Sanford, G. B. (1929). *Rep. Dom. Bot. Can.* for 1928, p. 167.

Turner, E. M. (1940). *Ophiobolus graminis* Sacc. var. *avenae*, var. n., as the cause of take-all or whiteheads of oats in Wales. *Trans. Brit. mycol. Soc.* **24**, 269.

Winter, A. G. (1939). Der Einfluss der physikalischen Bodenstruktur auf den Infektionsverlauf bei der Ophiobolose des Weizens. *Z. PflKrankh.* **49**, 513.

(*Received* 19 *May* 1941)

THE COLONIZATION OF BURIED WHEAT STRAW BY SOIL FUNGI, WITH SPECIAL REFERENCE TO *FUSARIUM CULMORUM*

By ALAN G. WALKER, Ph.D.

Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts

(With 11 Text-figures)

The saprophytic life of *Fusarium culmorum* has, until recently, been little studied, although its association with brown foot rot of cereals in both the seedling blight and whiteheads condition has long been established. *F. culmorum* has also been found in association with *Ophiobolus graminis* on plants attacked by the latter organism (Bennett, 1928; Schaffnit, 1930; Guyot, 1934; Sadasivan, 1939). More recently the presence of *Fusarium culmorum* on the roots and crowns of apparently healthy wheat plants has been recorded by Samuel & Greaney (1937), who isolated the fungus from surface-sterilized roots and crown pieces of normal plants which showed no disease symptoms. Three fields in different localities were sampled at fortnightly intervals between heading and harvest; in two of the fields the percentage of *F. culmorum* isolated increased with advance of the season, reaching a maximum in the isolations made from the stubble after harvest. This called attention to the earlier work of Broadfoot (1934), who obtained 20–60% of *F. culmorum* in isolations from many thousands of wheat plants taken at random from a series of rotation plots. Samuel & Greaney concluded that the fungus must have been present in the soil and entered the root only as vitality was lost after flowering, causing no appreciable damage, and that there was a further development of the fungus on the stubble after the crop had been cut.

Sadasivan (1939), who reviewed previous investigations on *F. culmorum*, studied the succession of soil fungi colonizing buried wheat straw. Natural unsterilized wheat straws, and sterilized straws treated with a 2% solution of sodium nitrate, were buried in a series of soils of various types. Samples of the straws were taken at regular intervals, surface-sterilized and plated out on potato-dextrose agar. Sadasivan found that *F. culmorum* developed on straws taken from all the soils examined and that *F. culmorum*, *Penicillium* spp. and *Mucor* spp. were numerically the most important soil fungi colonizing the straws. *Fusarium culmorum* and *Mucor* spp. were dominant in the early stages of colonization, being replaced by *Penicillium* spp. in the later stages of decomposition; the previous sterilization and nitrogenous treatment of the straws appeared to favour the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. Isolates of *Fusarium culmorum* thus obtained as saprophytes on wheat straw were shown to be pathogenic to wheat seedlings. On the basis of these observations, Sadasivan provisionally placed *F. culmorum* in Reinking & Manns' (1933, 1934) group of *soil inhabitants*, or *true soil fungi*.

The work described here was undertaken to confirm Sadasivan's conclusions concerning the saprophytic activity of *F. culmorum* and to find out whether the colonizing activity of

F. culmorum and other soil fungi fluctuated with the season. Sadasivan observed that soils collected in the autumn tended to give a higher proportion of *F. culmorum* and *Mucor* spp., and a lower proportion of *Penicillium* spp., than those collected in the spring. This suggested that *Fusarium culmorum* might reach a peak of activity in the soil in the autumn, after the ploughing in of the cereal stubble, and that later on in the following spring this fungus might be replaced, both in the straw and in the adjacent soil, by *Penicillium* spp.

Experimental

(*a*) *Colonization of wheat straw by soil fungi*

Experimental methods. The technique employed in these experiments on the fungal colonization of buried straw was essentially the same throughout, and hence a general description will serve, with modifications, for all the experiments to be described. The method used by Sadasivan (1939) was followed, sterilized straw being used for all experiments. The straw was selected from the internodes of healthy wheat plants and cut into pieces about 1 in. long. After soaking in tap water for 18 hr. the straws were drained and autoclaved for 30 min. at 1 atm. The straws were potted immediately after collection of the soil from the field, with as little air drying of the soil as possible. Sterilized linen bags were used for the collection of the soils, which, while in the bags, were broken up and well mixed, the larger stones and pieces of plant material being removed from each sample. The autoclaved straws were buried in between layers of soil in 3½ in. flower pots (size 60). In Exp. 1 only 12 straws, in 3 layers of 4, were buried in each pot; in the remaining experiments 25 straws, in 5 layers of 5, were buried per pot. The pots were watered after filling, and stood over a layer of moist sand in a lightly covered wooden box in the laboratory, being subsequently watered as necessary. The temperature of the laboratory seldom varied outside the range of 15–20° C., and the weekly mean temperatures were always within this range.

After the requisite period of incubation a sample of pots from each experimental series was taken and the straws washed over a sieve. The straws from each pot were kept together and surface-sterilized by shaking with an appropriate reagent in a rubber-stoppered tube, followed by washing in four changes of sterilized tap water. The straws were plated in the pure culture room on acidified (*p*H 5·0) potato-dextrose agar, four straws to each plate. The plates were incubated at 25° C., and the fungal colonies developing from each straw over a period of 14 days recorded. Frequently more than one and sometimes up to five colonies developed from each straw.

To simplify the presentation of data, only the numerically dominant species and genera have been tabulated; these are *Fusarium culmorum, Penicillium* spp., *Mucor* spp. and *Trichoderma* spp. Remaining fungal colonies were recorded as 'other fungi'. Results have been expressed graphically throughout. *The number of colonies belonging to each of the four groups has been expressed as a percentage of the total number of straws plated out*; the number of straws giving one or more fungus colonies on the plate has also been expressed separately.

In some samplings, plates were overrun by *Rhizopus* contaminations; such plates had generally to be discarded, which in effect reduced the size of the sample of straws taken. This *Rhizopus* contamination was found to be particularly troublesome in August, both in 1939 and 1940.

Exp. 1. In this experiment soil samples were taken systematically from nine of the plots and fields on the Experimental Station farm in each month of the year (1938–9) except January. The Rothamsted soil is a flinty clay loam; the nine different experimental plots or fields selected for sampling were as follows: Agdell Plot 1 (wheat after fallow in four-course rotation); Agdell Plot 2 (wheat after clover in four-course rotation); Great Harpenden, west half (wheat after beans); Great Harpenden, east half (wheat after clover); Broadbalk four plots (fallow after 4 years wheat and wheat after 1 year fallow, respectively; 86 lb. nitrogen/acre applied as sulphate of ammonia and as sodium nitrate, respectively), and Stackyard (pasture). The soil samples were taken at the mid-period of each month from September 1938 to September 1939 inclusive; the batch of plates from the August 1939 sampling was spoilt by contamination with *Rhizopus*.

The method of sampling was as follows: a convenient sampling line was adopted for each field or plot at the first sampling and adhered to at subsequent monthly samplings. The first sample was taken six paces into the field or plot, and the other samples at six-pace intervals along the line. The soil samples were therefore taken from approximately the same sites at each sampling. Ten samples

were taken per plot; each sample, comprised of four trowels full of soil taken at arm's length to the front, right, rear and left, respectively, was placed in a sterilized bag. The trowel was not sterilized but was merely wiped clean between the taking of consecutive samples.

The samples were potted separately, and the pots incubated for 14 days before the washing out and plating of the straws. Surface sterilization was effected in this experiment by shaking for 1–1½ min. in 1 in 1000 aqueous mercuric chloride, followed by washing in four changes of sterile tap water.

Fig. 1 shows the relationship of the different groups of fungi to one another when the results from all nine sampling areas are averaged. *Fusarium culmorum* was the numerically dominant organism colonizing the straws throughout the year, followed by the group of *Penicillium* spp., whilst *Mucor* spp. and *Trichoderma* spp. remained at a constantly lower level than the first-named through most of the year. The proportion of *Fusarium culmorum* colonies fluctuated, but it is difficult to attach any special significance to these fluctuations; the same is true of the relative numbers of *Trichoderma* spp., which remained at a lower level throughout. The proportion of *Mucor* colonies was higher in the two September samplings than in any other month, whilst the proportion of *Penicillium* was lowest in the two September samplings.

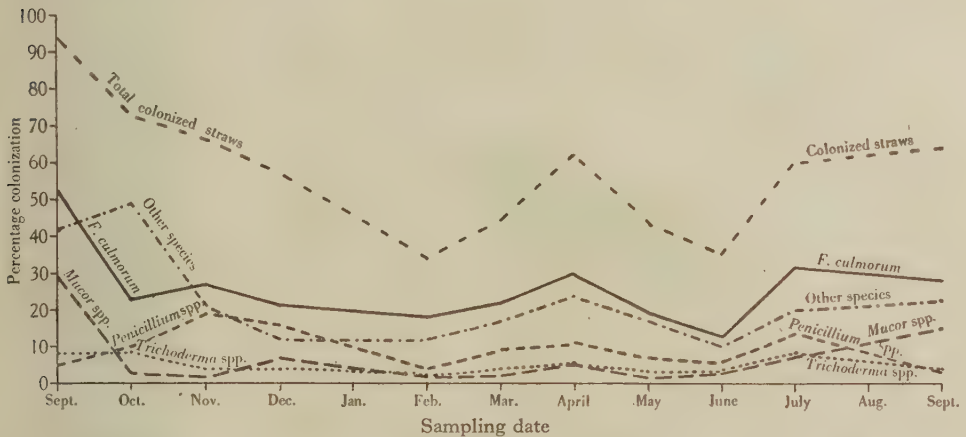


Fig. 1. Development of fungi from straws buried in the nine soils of Exp. 1.

Fig. 1 also shows the percentage of straws yielding fungus colonies throughout the year as a mean of all plots; this affords a measure of fungus activity in straw colonization. As might have been expected, the curve falls to a minimum in February and rises to a second maximum in September. An unexpected depression occurs at the May and June samplings; this may be due to the heavy rain occurring just before sampling days in these two months, which led to a high soil moisture content, even reaching saturation in some plots.

The variation in fungal colonization of the straws in the different sampling areas is shown in Fig. 2, in which the figures for the whole sampling period are averaged for each area. *Fusarium culmorum* was highest in Stackyard (grass) and in Plot 16 (wheat and fallow, sulphate of ammonia) of Broadbalk, next in Plot 7 (wheat and fallow, nitrate of soda) of Broadbalk and in Great Harpenden (wheat after beans and clover respectively) and lowest in Agdell (wheat after fallow and clover, four-course rotation). The numbers of this fungus did not appear to be influenced by the fallowing policy on Broadbalk, but were affected by

the form of nitrogen applied. *Penicillium* spp. was the group next in importance to *Fusarium culmorum*, but numbers did not vary much from field to field. *Mucor* spp. and *Trichoderma* spp. were the least important, except in Agdell field under the four-course rotation, in which all four groups were equally important.

The pathogenicity of sixty-five isolates of *Fusarium culmorum* obtained in this experiment at different times of the year and from the nine different sampling areas was tested on wheat seedlings in pots of sand. The pathogenicity of these isolates, estimated by Shen's (1940) disease rating, compared favourably with that of two isolates of *F. culmorum* obtained from diseased plants, which were included in the test.

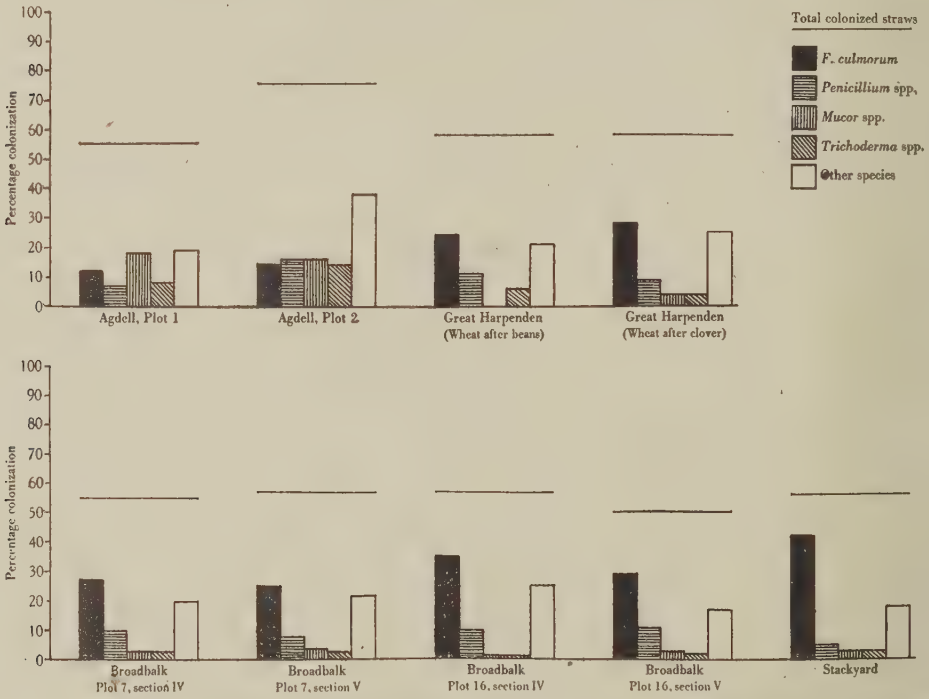


Fig. 2. Development of fungi from straws buried in soil from the nine sampling areas of Exp. 1, averaged for each area for the whole sampling period.

Exp. 2. In this experiment, the buried straw technique was employed with sixteen different soils collected from widely separated parts of the country. The soils were collected in the early part of August 1939 and stored in the sample bags until the experiment was set up in October. The different soils are described below, *p*H values being determined by the quinhydrone electrode:

(1) *Helpston.* A medium loam collected in Northamptonshire, *p*H 8·01. Wheat 1938–9.
(2) *Newark.* Clay-loam collected near Peterborough, *p*H 6·32. Wheat 1938–9.
(3) *Crowland.* Black fen soil from Lincolnshire, *p*H 7·30. Wheat 1938–9.
(4) *Normanton.* Loam from Rutland, *p*H 7·89. Wheat 1938–9.
(5) *Beenham.* Loam, overlying the chalk, from Berkshire, *p*H 6·87.
(6) *Lambourn.* Loam, overlying the chalk, from Berkshire, *p*H 7·66. Oats 1938–9.
(7) *Cholsey.* Loam, overlying the chalk, from Berkshire, *p*H 7·66. Wheat 1938–9.
(8) *Lockford.* Loam, overlying the chalk, from Hampshire, *p*H 7·87. Wheat 1938–9.
(9) *Cambridge.* Calcareous loam from Cambridge University Farm, *p*H 7·94. Wheat 1938–9, undersown with clover.

(10) *Duxford.* Calcareous loam from Cambridgeshire, *p*H 7·87. Wheat 1938–9.
(11) *Knebworth.* Clay-loam from Hertfordshire, *p*H 7·61. Wheat 1938–9.
(12) *Brynamlwg.* Medium loam from Cardiganshire, *p*H 5·47. Wheat 1938–9.
(13) *Nancellan.* Clay-loam from Cardiganshire, *p*H 6·52. Oats 1938–9.
(14) *Nanllan.* Clay-loam from Cardiganshire, *p*H 6·09. Wheat 1938–9.
(15) *Cwm I.* Loam over shale from Cardiganshire, *p*H 5·27. Oats 1938–9.
(16) *Cwm II.* Loam over shale from Cardiganshire, *p*H 5·51, collected from the vicinity of Cwm I but ploughed up from waste land. July 1939.

Four pots were filled from each sample of soil; twenty-five sterilized straws being buried in each, and the pots incubated for 28 days. In this experiment the washed straws were surface-sterilized by shaking for 1–1½ min. in 1 in 1000 aqueous mercuric chloride and then plated out as usual on acidified potato-dextrose agar.

The results are given in Fig. 3; the different soils are arranged in order of decreasing importance of *F. culmorum*, which was dominant in only four soils, viz. Newark, Normanton, Helpston and Crowland. In the other twelve soils, *Penicillium* spp. was the dominant group of fungi. The inverse relationship between *Fusarium culmorum* and *Penicillium* spp. is the most interesting feature of this experiment. Too much importance should not be attached to differences in straw colonization in these sixteen soils, on account of differences in soil moisture content at the time of collection, and also in the rate of drying out of the soil in the bags. Fig. 3 also shows that in no soil did the percentage of straws colonized fall below 80. The number of genera of fungi identified on the straws in this experiment was meagre; *Acrostalagmus* spp. were found on the straws from thirteen soils, *Aspergillus* spp. from three soils, *Fusarium* spp., other than *Fusarium culmorum*, from three soils, *Gliocladium* spp. from five soils, *Stemphyllium* sp. from one soil, and a *Pythium*(?) sp. from four soils. Many of the 'other fungi' had to remain classed as 'sterile mycelia'.

(*b*) *Pathogenicity test of* F. culmorum *isolates from Exp.* 2

Isolates obtained in this experiment from eleven of the sixteen sterilized soils were tested for pathogenicity against six isolates of the fungus obtained from diseased cereal plants, viz.:

Isolate P.A. from oats.	Isolate P.D. from barley.
Isolate P.B. from wheat.	Isolate P.E. from wheat.
Isolate P.C. from maize.	Isolate P.F. from wheat.

In this test unsoaked Little Joss wheat seed was inoculated by steeping for 20 min. in a spore suspension of 20,000 per c.c.; the suspensions were obtained from 10 weeks old bottle slants of the different isolates on potato-dextrose agar, and were all adjusted to the above spore density. Seven inoculated seeds were planted in each pot of silver sand, five pots being allotted to each isolate. A control series of sixteen non-inoculated pots was planted at the same time. Before planting the pots of sand were watered once with nutrient solution. The experiment was carried out in the glass-house in March 1940; the plants were washed out and the degree of infection recorded by Shen's (1940) method after 21 days (Table 1).

The percentage emergence in the sixteen control pots was 95; only 5/112 plants showed any root infection and the disease rating of these control plants was only 0·8. The results of this experiment show once more that the 'saprophytic' isolates of *Fusarium culmorum* from buried straws are no less pathogenic to wheat seedlings than isolates obtained directly from diseased cereal plants.

Exp. 3. In the preceding experiments a negative correlation between the occurrence of *F. culmorum* and that of *Penicillium* spp. in the different soils was suggested (Fig. 3);

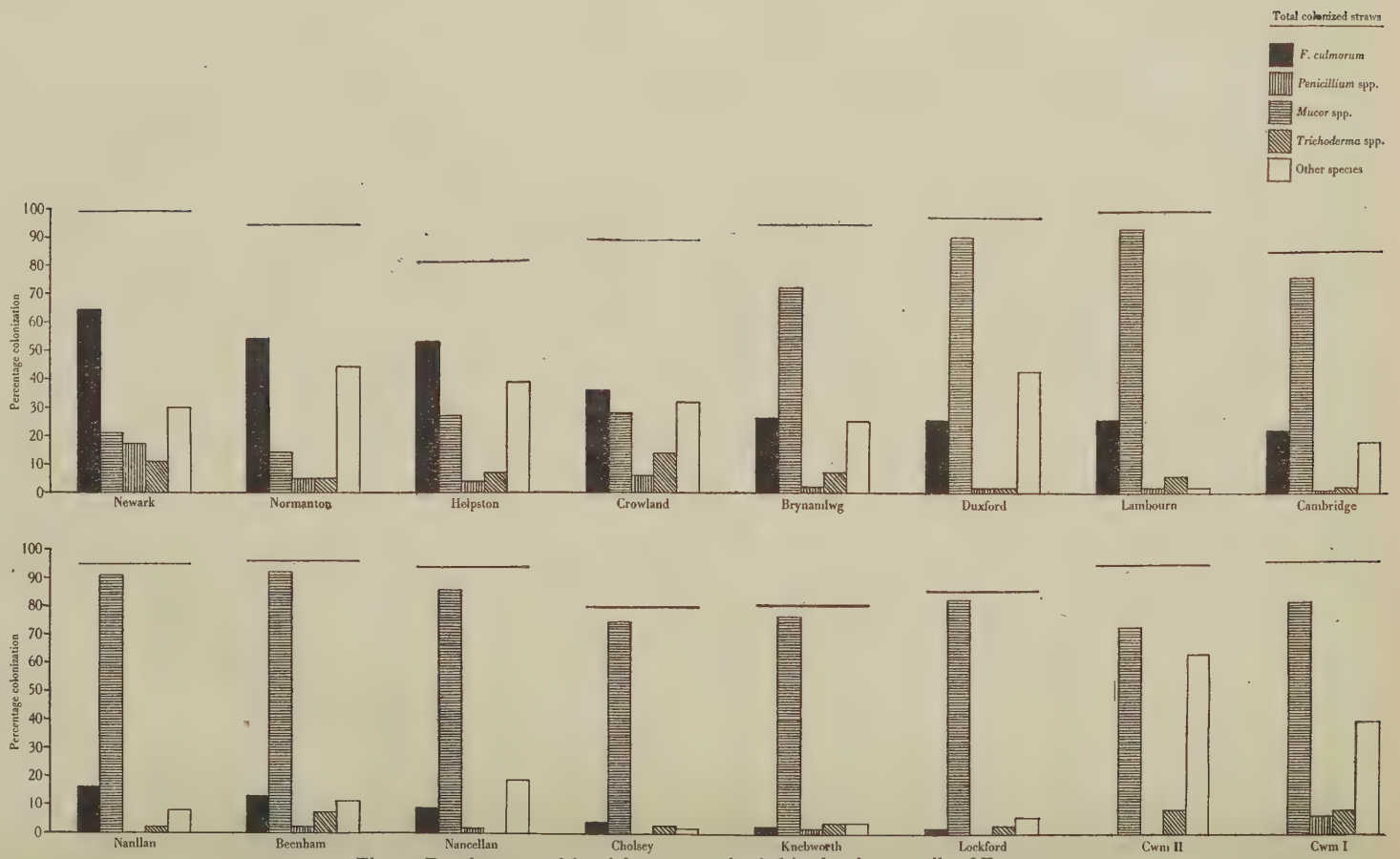


Fig. 3. Development of fungi from straws buried in the sixteen soils of Exp. 2.

confirmation on a more extensive scale of Sadasivan's observations on the replacement of *Fusarium culmorum* by *Penicillium* spp. in the buried straws at a later stage was felt to be needed. Accordingly an experiment on the succession of fungi in straws buried in three different soils was planned, the samplings to be spread over a period of some 6 months.

TABLE 1. *Infection of wheat seedlings by isolates of* Fusarium culmorum *from Exp.* 2

Isolate	Infected seedlings	Pre-emergence killing	Post-emergence killing	Disease rating
Helpston	23	2	1	27
Crowland	25	1	1	24
Newark	20	2	0	20
Normanton	28	1	2	30
Beenham	20	1	0	18
Lambourn	24	2	2	26
Brynamlwg	17	1	1	17
Nancellan	15	2	1	19
Cambridge	24	4	1	30
Duxford	21	3	2	28
Nanllan	21	4	5	26
P.A.	19	2	1	23
P.B.	24	2	0	21
P.C.	29	3	2	36
P.D.	23	0	0	22
P.E.	22	3	1	22
P.F.	28	3	3	36

The soils were as follows:

(1) *Great Harpenden*, as in Exp. 1; the soil was collected from over the ploughed stubble of the previous season's (1939) wheat crop; the field had carried clover in 1938.

(2) *Harpenden allotment soil*, as used by Sadasivan in his experiments, but not from the identical site; collected from under weeds.

(3) *Woburn soil*, obtained from one of the arable fields at the Woburn Experimental Station.

All soils were collected in March 1940, immediately before the setting up of the experiment, and were passed through a $\frac{1}{4}$ in. sieve. As internodal straws disappear after less than 6 months in the soil, nodal straw of the type used by Garrett (1938) was employed. Twenty-five straws were buried in each pot; eight pots of each soil, giving 200 straws in all, were taken at every sampling. After washing, the straws were surface-sterilized by shaking for $1\frac{1}{2}$ min. in 1 in 1000 aqueous mercuric chloride. Samples were taken at 2 weeks, 4 weeks and thence at 4 weekly intervals to 20 weeks. Results are shown in Figs. 4–6.

The percentage of straws colonized by fungi in the three soils was consistently highest throughout the experiment in Great Harpenden soil and almost as consistently the lowest in Woburn.

Considering the results from the three soils together, Sadasivan's observations as to the paramount importance of *Fusarium culmorum* in the early, and *Penicillium* spp. in the later, stages of straw colonization were confirmed only in the Great Harpenden and Woburn soils. Allotment soil has given a different result in this experiment, although Sadasivan recorded 100% of *Fusarium culmorum* from this type of soil in one of his samplings. In all three soils of this experiment, *Mucor* spp. have fairly consistently declined from a maximum at the first and second samplings, whilst *Trichoderma* spp. have fluctuated but shown no very pronounced trend.

Exp. 4. The results of the first three experiments suggested that the type as well as the extent of fungal colonization might be influenced by the surface-sterilizing reagent employed, and by the time of treatment given. It was also possible that the type of colonization might

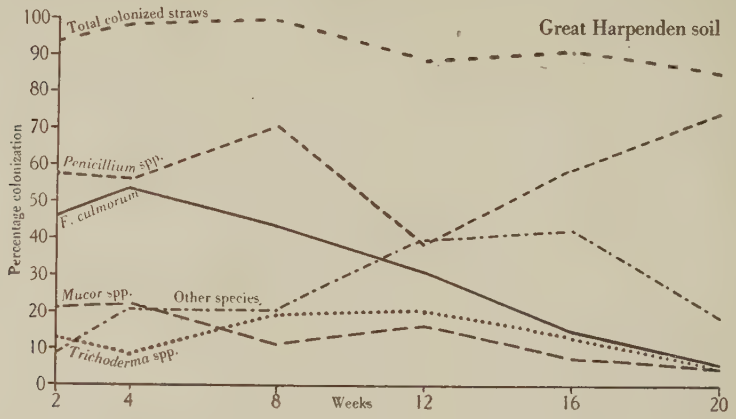


Fig. 4. Development of fungi from straws buried in Great Harpenden soil of Exp. 3.

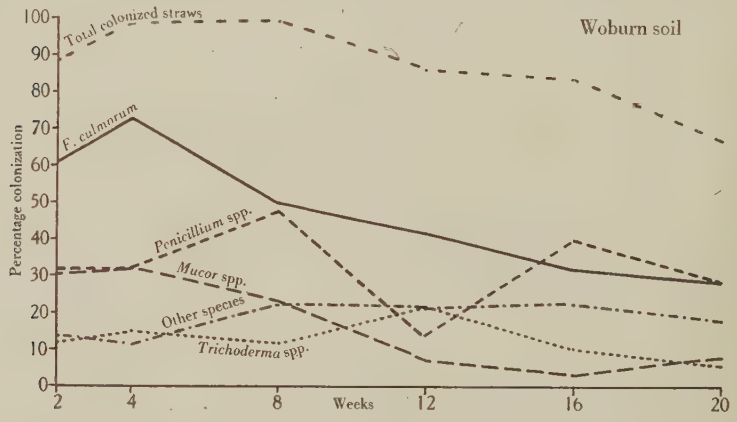


Fig. 5. Development of fungi from straws buried in Woburn soil of Exp. 3.

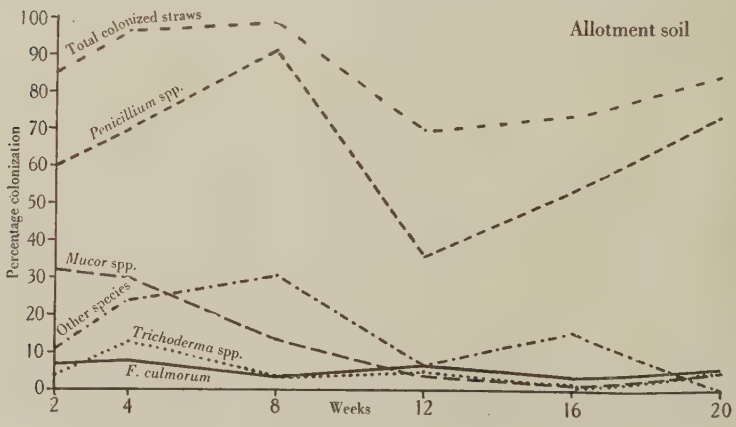


Fig. 6. Development of fungi from straws buried in allotment soil of Exp. 3.

be influenced by the culture medium employed and that a medium other than acidified potato-dextrose agar might give somewhat different results. In Exps. 1 and 2, the time of immersion in the 1 in 1000 mercuric chloride solution varied between 1 and 2 min., and it was thought that part of the inconsistencies in the results might be traced to this cause. In Exp. 3 the time of immersion in mercuric chloride was fixed as rigidly as possible at $1\frac{1}{2}$ min.

Different surface-sterilizing agents were compared by Mead (1933), both for the isolation of fungi from wheat roots, and for the production of sterile wheat seedlings by seed sterilization. Silver nitrate was found most satisfactory for seed sterilization, but its action was too strong for the isolation of pathogenic fungi; mercuric chloride proved as good for the first purpose, and more satisfactory for the second. Calcium hypochlorite was very satisfactory for root isolation, but for seed sterilization a long period of immersion was required. Hydrogen peroxide was only a mildly toxic agent and its strength soon depreciated. Washing with sterile water in Simmonds' (1930) washing machine was the most satisfactory method of treating young and delicate roots from which fungal isolations were required.

Davies (1935) compared mercuric chloride, silver nitrate, calcium hypochlorite and hydrogen peroxide at different concentrations and for different times of immersion as surface-sterilizing agents for the isolation of *Ophiobolus graminis* from young infected wheat stems. Silver nitrate was less toxic to *O. graminis* than mercuric chloride, and the frequency of isolation of this fungus was substantially higher with the first named sterilizing agent. With *Helminthosporium sativum*, on the other hand, silver nitrate was more toxic than mercuric chloride. These observations of Davies are of particular interest, inasmuch as they afford evidence of specificity in the action of sterilizing agents on these root infecting fungi.

The sterilizing agents used in the experiment described here were mercuric chloride, calcium hypochlorite and silver nitrate, which were employed as follows:

Mercuric chloride—as a 1 in 1000 aqueous solution for 2 and 4 min. periods of immersion, respectively, followed by washing in four changes of sterile tap water. This agent at 1 in 1000 strength was employed both by Mead (1933) and by Davies (1935).

Calcium hypochlorite—as a 1 in 14 suspension for 10 and 20 min. periods of immersion, respectively, followed by washing in six changes of sterile tap water. Mead and Davies both used a calcium hypochlorite suspension of the 1 in 14 strength, the former using a 20 min. period, and the latter a 2 and 5 min. period of immersion respectively.

Silver nitrate—the technique of Padwick (1938) was followed. Periods of 2 and 4 min., respectively, in 1% silver nitrate were followed by a brief immersion in saturated sodium chloride solution, followed by immediate plating out of the material on agar. The saturated sodium chloride was not heat sterilized but was prepared some time before use. In this method, the sterilizing agent, silver nitrate, is not washed away by sterile water, but is precipitated in the saturated sodium chloride solution as silver chloride, which is highly insoluble in water.

In setting up this experiment, the straws were buried in Great Harpenden soil, as collected in March 1940 for Exp. 3, with twenty-five straws per pot. Two samplings were made at 14 and 28 days, respectively, and at each sampling four pots (100 straws) were allotted to each of the six sterilization treatments. As usual, acidified potato-dextrose agar was used as the plating medium. The results of this experiment are given in Figs. 7 and 8.

Percentage colonization of the straws was greater after 28 days than after 14 days; at both samplings it was highest in the calcium hypochlorite series. At the 28-day sampling, the percentage colonization in the 2 min. silver nitrate series was lower than that in the 4 min. series.

Fusarium culmorum was the dominant colonist in the calcium hypochlorite series, both in the 10 and 20 min. treatments and at both 14- and 28-day samplings. Its percentage occurrence was depressed by the 2 min. treatments with silver nitrate and with mercuric chloride, respectively, and it was completely cut out by the 4 min. treatments with these two agents, except for a 2% occurrence in the 4 min. mercuric chloride treatment at 28 days.

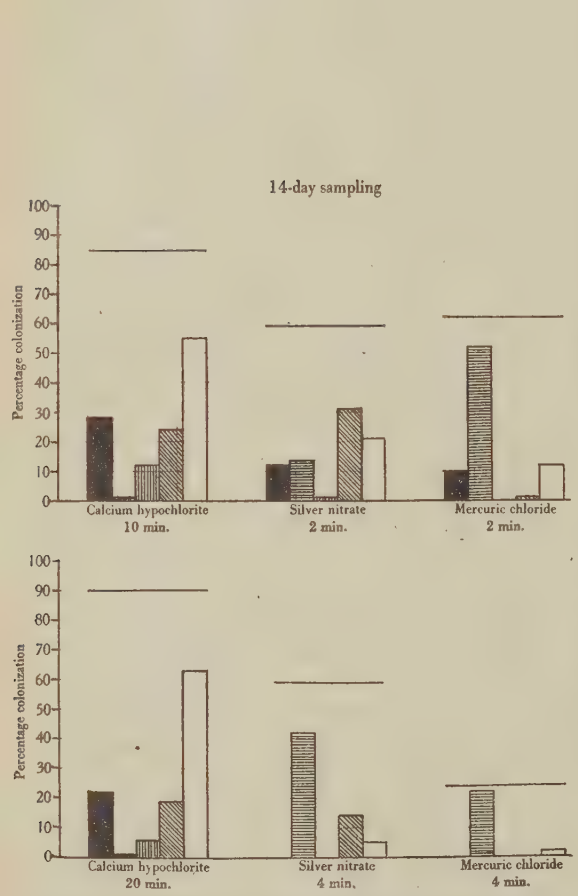


Fig. 7. Development of fungi from straws buried for 14 days in soil of Exp. 4, after treatment with different sterilizing agents.

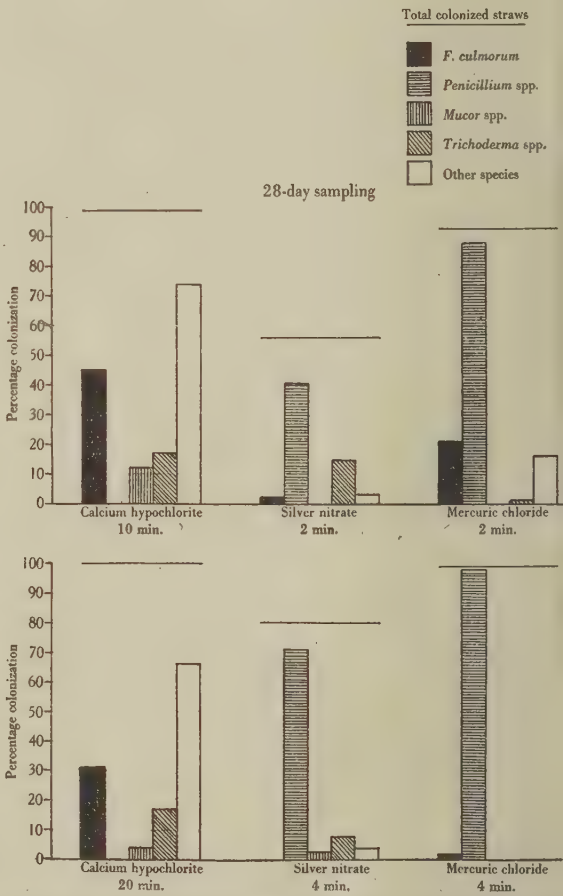


Fig. 8. Development of fungi from straws buried for 28 days in soil of Exp. 4, after treatment with different sterilizing agents.

Penicillium spp. were reduced to 1–2% occurrence by the calcium hypochlorite treatments at 14 days and to nil at 28 days. They were the dominant group in the silver nitrate and mercuric chloride treatments at both strengths and at both samplings with one exception, the 2 min. silver nitrate at 14 days, in which *Trichoderma* spp. were dominant. The relative ascendancy of *Penicillium* spp. was greater in the mercuric chloride than in the silver nitrate treatment and increased with the time of treatment from 2 to 4 min.

Mucor spp. were highest at the 10 min. treatment with calcium hypochlorite, were lower

in the 20 min. treatment with this agent, occurred only twice, at and below the 3 % level, in the silver nitrate series, and not at all in the mercuric chloride series.

Trichoderma spp. appeared to be particularly tolerant of silver nitrate and were the dominant fungi in the 2 min. treatment at 14 days. The 4 min. treatment with this agent, however, suppressed *Trichoderma* spp. more than the calcium hypochlorite treatments, in which percentage occurrence of *Trichoderma* spp. was second only to *Fusarium culmorum* throughout both treatments and samplings. Mercuric chloride appeared to be particularly toxic towards *Trichoderma* spp., reducing them to a 2 % occurrence at the 2 min. treatment and to nil at the 4 min. treatment at both samplings.

Thus, calcium hypochlorite secured the most even distribution of colonies amongst the four groups, with *Fusarium culmorum* as a dominant; silver nitrate favoured the dominance of *Penicillium* spp., with *Trichoderma* spp. markedly subdominant; mercuric chloride greatly simplified the fungus flora of the plates, which consisted in the 2 min. treatment largely, and in the 4 min. treatment almost entirely, of *Penicillium* spp. The results are obviously not to be interpreted on the sole basis of specific toxicity of the different agents towards the four different groups of fungi, but rather through the selective action of the agents on the struggle for dominance on the agar medium between the different fungi in the straw. Calcium hypochlorite appears to be a mild sterilizing agent, and the fungus flora of *Fusarium culmorum*, *Trichoderma* spp. and *Mucor* spp. developing from the straw in this treatment is characterized by speed and density of growth rather than by high resistance to fungicidal action. Mercuric chloride, on the other hand, appears to be a particularly severe sterilizing agent and greatly simplifies the flora, which is reduced chiefly to *Penicillium* spp., characterized by relatively slow growth but high resistance to the fungicide. Silver nitrate appears to occupy a position intermediate in this respect between calcium hypochlorite and mercuric chloride.

One feature of the results calls for comment. The proportion of *Fusarium culmorum* to *Penicillium* spp. is much lower in the 2 min. treatment with mercuric chloride than in Exps. 1, 2 and 3 when using this surface-sterilizing agent. Times of sterilization in the preceding experiments were, however, shorter, being 1–1½ min. in Exps. 1 and 2 and a standardized 1½ min. in Exp. 3. An increase in the time to 2 min. in Exp. 4 has certainly much increased the *Penicillium* spp. at the expense of *Fusarium culmorum*. It seems likely, therefore, that part of the unexplained variation in the results of Exp. 1 could have been eliminated by more careful standardization of the time of immersion in the mercuric chloride.

Exp. 5. This experiment was planned to include a study of fungal colonization not only of sterile straws, but also of straws inoculated with, and completely permeated by, pure cultures of *Fusarium culmorum* and *Penicillium* sp., when subsequently buried in the soil.

The inoculated straws were prepared by a modification of Garrett's (1938) method. The *Fusarium culmorum* isolate was obtained from Stackyard soil during the course of Exp. 1; the *Penicillium* sp. from Great Harpenden soil, the isolate being taken from the series treated for 4 min. with mercuric chloride in Exp. 4.

The soil was taken from an outside stack of Great Harpenden soil, collected from the field in March 1940 and employed in Exps. 3 and 4, and subsequently in this experiment some 2 months later. The usual twenty-five straws were buried in each pot; four pots, comprising 100 straws, were taken from each series at each sampling, at 14, 28 and 56 days, respectively.

The three surface-sterilizing treatments used in Exp. 4 were employed with one additional treatment, viz.

Sterile water. Washing in six changes of sterile tap water.
Calcium hypochlorite, treatment for 10 min.
Silver nitrate, treatment for 2 min.
Mercuric chloride, treatment for 2 min.

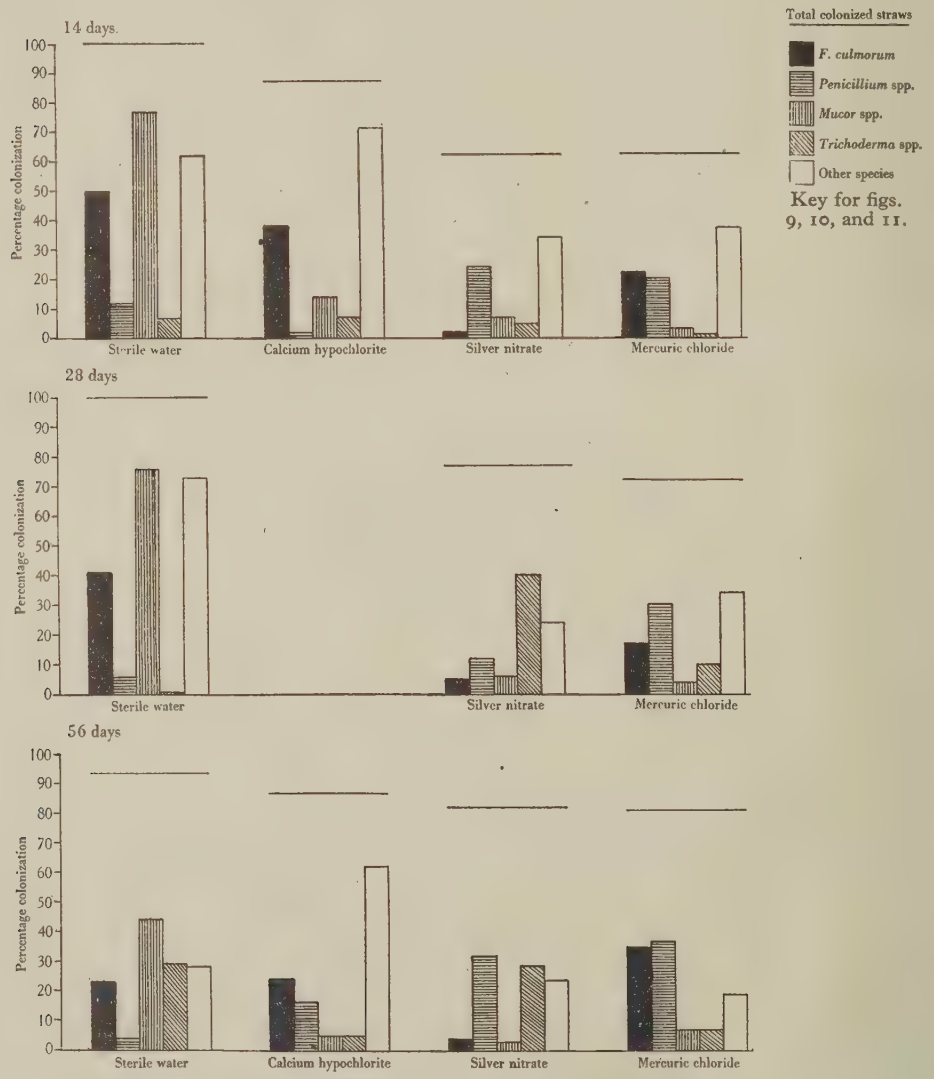


Fig. 9. Development of fungi from originally sterile straws buried in soil of Exp. 5, after treatment with different sterilizing agents.

Unfortunately both the originally sterile, and the *Penicillium*-inoculated series of straws treated with calcium hypochlorite at the 28-day sampling were spoilt through an accident in handling, and results for these two series are consequently lacking. The results of this experiment are given in Figs. 9–11.

Originally sterile straws (Fig. 9). The percentage colonization of straws by fungi was 100 in the 14- and 28-day samplings, and 93 in the 56-day sampling of the sterile water series, was somewhat reduced in the calcium hypochlorite series and still further reduced in the silver nitrate and mercuric chloride series.

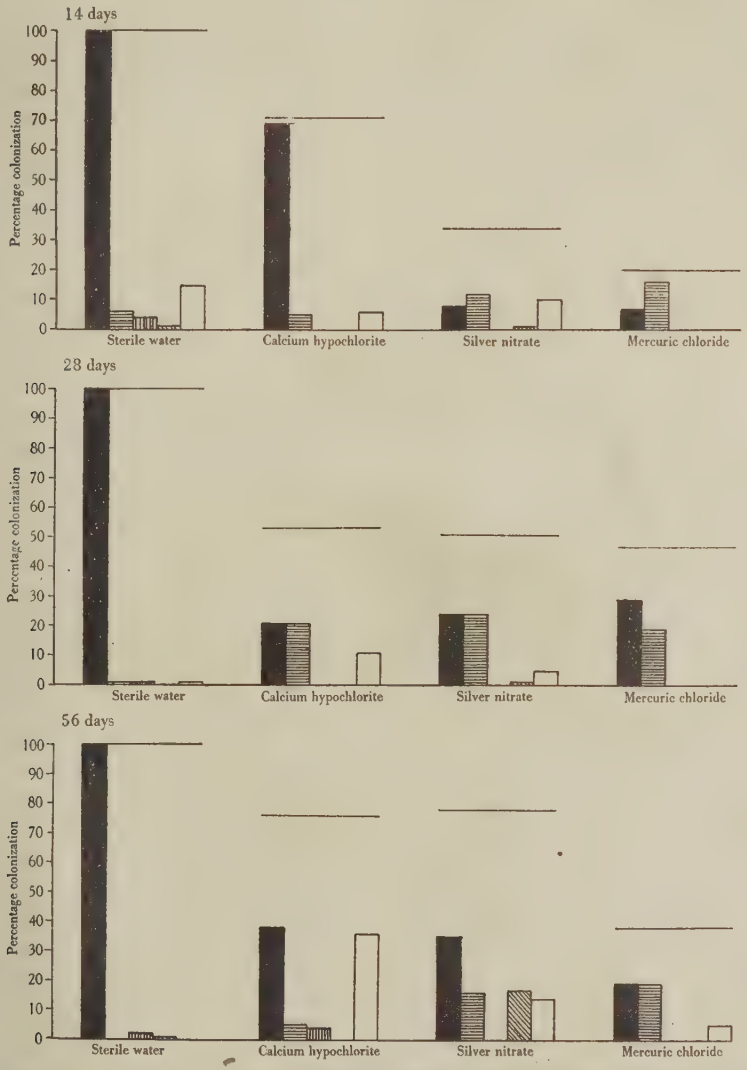


Fig. 10. Development of fungi from straws, originally inoculated with *Fusarium culmorum*, buried in the soil of Exp. 5, after treatment with different sterilizing agents.

Fusarium culmorum was subdominant to *Mucor* spp. in the 14- and 28-day samplings of the sterile water series and surpassed both by *Mucor* spp. and by *Trichoderma* spp. in the 56-day sampling. As in Exp. 4 it was dominant in the calcium hypochlorite series. In the silver nitrate series it was the lowest of all four groups of fungi in the 14- and 28-day samplings

and lowest but one in the 56-day sampling. Taking the three samplings of the mercuric chloride series together, *Fusarium culmorum* might be classed as co-dominant with *Penicillium* spp. in this series.

Penicillium spp. were lower in the sterile water series than in the calcium hypochlorite

Fig. 11. Development of fungi from straws, originally inoculated with *Penicillium* sp., buried in the soil of Exp. 5, after treatment with different sterilizing agents.

series, and under both series were poorly represented. The poor development of *Penicillium* spp. in the sterile water series supports the suggestion advanced above that their sparse development in the calcium hypochlorite series in Exp. 4 was due less to any specific toxicity of the agent to *Penicillium* spp. than to the competition of more vigorously growing

species, which calcium hypochlorite had failed to suppress. In the three samplings of the silver nitrate series, *Penicillium* spp. were first dominant, then subdominant to *Trichoderma* spp. and finally co-dominant with that group. After the mercuric chloride treatment, *Penicillium* spp. appeared co-dominant with *Fusarium culmorum*.

Mucor spp. were dominant in the sterile water series, were displaced by *Fusarium culmorum* and later also by *Penicillium* spp. in the calcium hypochlorite series, and were relatively unimportant in the silver nitrate and mercuric chloride series.

Trichoderma spp. remained at a relatively low level in all series except in the last sampling of the sterile water series, when they were all subdominant to *Mucor* spp., and in the mid and last samplings of the silver nitrate series, when they were absolutely dominant and then co-dominant with *Penicillium* spp., respectively. As in Exp. 4, *Trichoderma* spp. appeared to be peculiarly tolerant of silver nitrate.

Straws originally inoculated with Fusarium culmorum (Fig. 10). The 4 weeks' incubation in the pure culture flasks at 25° C. after inoculation with *F. culmorum* and before burial in the soil seemed to have a remarkable effect upon the percentage colonization of the straws by fungi. Whilst this was 100% (chiefly *F. culmorum*) in the series treated with sterile water, it was reduced to the 50–75% level by calcium hypochlorite and still further by the silver nitrate and mercuric chloride treatments. In the two latter treatments percentage colonization increased in the mid and last samplings, especially in the case of the silver nitrate series. The effect of the sterilizing agents on percentage colonization may be partially explained by supposing that other fungi, more resistant to the sterilizing agents, had not established themselves sufficiently in the straws to take the place of the killed *F. culmorum* on the isolation plates. This suggestion is supported by the increase in the percentage colonization of the straws at the two latter samplings in the silver nitrate and mercuric chloride series. At the same time this increase is made up as much by an increase in the number of *F. culmorum* colonies as by an increase in the number of *Penicillium* spp. supposedly more resistant to the fungicides. The severe depression in fungal colonization can, therefore, be but partially explained by this suggestion.

Straws originally inoculated with Penicillium *sp.* (Fig. 11). Colonization of the straws was 100% throughout in the sterile water series and in the mercuric chloride series; in the three samplings of the silver nitrate series it was 100, 90 and 97% respectively. The percentage occurrence of *Penicillium* spp. also remained at almost 100 throughout these three series, although a number of the straws yielded other colonies as well, particularly in the sterile water series. An interesting feature of the *Penicillium* inoculated straws was the marked depression both in percentage straws colonized by fungi, and in the percentage colonized by *Penicillium* in the calcium hypochlorite treatment at the 56-day sampling. A smaller depression can be observed in the 14-day sampling.

Summarizing the results of Exps. 4 and 5, upon the action of different surface-sterilizing agents upon the straws originally sterile when buried, the numerically largest, and also the most varied fungus floras were observed to grow out from the straws washed in sterile water alone. *Mucor* spp. were dominant, with *Fusarium culmorum* tending to be subdominant. Calcium hypochlorite appeared to be a mild sterilizing agent which resulted in a comparatively large and varied fungus flora growing out from the straws, with *F. culmorum* tending to be dominant. This agent appeared to have a specifically toxic effect upon *Penicillium* spp., and the latter were probably further suppressed by the more vigorous

growth of other species relatively tolerant of calcium hypochlorite. Silver nitrate reduced and simplified the fungus flora derived from the straws; *Penicillium* spp. tended to be dominant, but were sometimes replaced by *Trichoderma* spp., which appeared specifically tolerant of this sterilizing agent. Mercuric chloride, especially in the longer treatment, also tended to reduce and simplify the fungus flora obtained from the straws; with shorter times of treatment *Fusarium culmorum* might be dominant, but as length of treatment increased *Penicillium* spp. increased until they might become the sole fungus obtained from the straws. *Penicillium* spp. therefore appeared to be particularly tolerant of mercuric chloride.

Discussion

In the experiments described, the importance of *Fusarium culmorum* as a colonizer of buried wheat straw has been demonstrated in a number of cultivated soils, at all seasons of the year. Sadasivan's (1939) hypothesis that the relative colonizing activity of *F. culmorum* and other soil fungi might fluctuate according to the season was not confirmed by the experiments; nevertheless, it must not be concluded that no such fluctuation occurs. Subsequent experiments have demonstrated that the growth of fungus colonies from the straws, in addition to being affected by the period of incubation of the straws in the soil, is dependent on the surface-sterilizing agent used and the time of immersion in that agent; thus, had the period of immersion been more strictly controlled in Exp. 1, it is possible that some of the unexplained variations in colonization would have been eliminated. Sadasivan's conclusions as to the succession of fungi on buried wheat straw have been confirmed only in part.

The experiments have demonstrated the equal importance of the group of *Penicillium* spp. as colonizers of buried straw, and have indicated an inverse relationship between the occurrence of *Penicillium* spp. and *Fusarium culmorum* on the plates. The struggle for dominance between *Penicillium* spp. and *Fusarium culmorum* in the buried wheat straw is evidently affected by the process of surface sterilization, which may actually reverse the final issue of the struggle when the straws are plated out (Exps. 4 and 5).

The saprophytic isolates of *Fusarium culmorum* from buried wheat straw have proved to be just as pathogenic to wheat seedlings as those isolated from diseased plants; straws invaded by *F. culmorum* as a saprophyte may therefore form potential centres for infection of the underground parts of cereal plants coming into contact with them, as well as a means of perpetuation of the organism in the soil.

In this connexion particular interest attaches to the observations of Russell (1934) in Saskatchewan and of Sanford (1939) in Alberta that whereas the incidence of take-all (*Ophiobolus graminis*) of wheat was markedly reduced by crop rotation, that of common rot (*Fusarium culmorum* and *Helminthosporium sativum*) was scarcely affected. Tyner (1940) has investigated the effect of adding wheat, oat and barley straw, respectively, upon the development of disease in successive crops of wheat seedlings grown in pots originally inoculated at seed-level with cultures of *Fusarium culmorum*, *Helminthosporium sativum* and *Ophiobolus graminis*, respectively. Whilst wheat straw tended to be the most, and oat straw the least, favourable to the development of disease in the seedlings, the effects were not consistent in successive plantings, nor could any relation be established between the development of disease and the amount of straw added.

Summary

A study has been made by Sadasivan's (1939) technique of the colonization by fungi of wheat straw buried in the soil. One-inch lengths of sterilized straw were buried in the experimental soils in $3\frac{1}{2}$ in. flower pots; after incubation at laboratory temperature (16–20° C.) for the required period the straws were washed out of the soil, surface sterilized by mercuric chloride or other sterilizing agent and plated out on acidified potato-dextrose agar (*p*H 5·0).

Fusarium culmorum and *Penicillium* spp. were numerically the most important organisms developing from the straws on the plates, at least during the first 5 months of incubation in the soil. Both groups of organisms, together with others, appeared generally to be present in the decomposing straw, but the method of surface sterilization employed apparently decided which organism produced a colony on the isolation plate.

Fusarium culmorum, a fungus of a vigorous and rapid habit of growth, showed low resistance to the action of the more severe sterilizing agents, such as mercuric chloride and silver nitrate, but developed better after surface sterilization of the straws with calcium hypochlorite, a mild sterilizing agent, and best of all after a mere washing in sterile water. *Penicillium* spp. were apparently crowded out by the more vigorous growth of *Fusarium culmorum* after these mild treatments of the straws; on the other hand, they were very tolerant of the more severe surface sterilizing agents, mercuric chloride and silver nitrate, and after the longer period of treatment were often the only organisms developing on the plates.

The pathogenicity to wheat seedlings of the isolates of *F. culmorum* obtained from decomposing wheat straw was shown to be comparable with that of isolates of the same fungus secured from diseased cereal plants.

I am indebted to Mr S. D. Garrett for suggesting this problem, and for his interest and helpful criticism throughout the course of the investigations. Thanks are also due to Miss M. D. Glynne for assistance in the identification of some of the genera mentioned. A portion of the work was carried out during my tenure of an Agricultural Research Scholarship.

REFERENCES

Bennett, F. T. (1928). On two species of *Fusarium*, *Fusarium culmorum* (W. G. Sm.) Sacc. and *Fusarium avenaceum* (Fries) Sacc. as parasites of cereals. *Ann. appl. Biol.* **15**, 213.

Broadfoot, W. C. (1934). Studies on foot and root rot of wheat. IV. *Canad. J. Res.* **10**, 115.

Davies, F. R. (1935). Superiority of silver nitrate over mercuric chloride for surface sterilization in the isolation of *Ophiobolus graminis* Sacc. *Canad. J. Res.* Sect. C, **13**, 168.

Garrett, S. D. (1938). Soil conditions and the take-all disease of wheat. III. *Ann. appl. Biol.* **25**, 742.

Guyot, A. L. (1934). Observations sur quelques maladies fusariennes des céréales en France. *Rev. Path. veg.* **21**, 143.

Mead, H. W. (1933). Studies of methods for the isolation of fungi from wheat roots and kernels. *Sci. Agric.* **13**, 304.

Padwick, G. W. (1938). Complex fungal rotting of pea seeds. *Ann. appl. Biol.* **25**, 100.

Reinking, O. A. (1934). Parasitic and other *Fusaria* counted in Costa Rica and Panama soils. *Zbl. Bakt.* Abt. 2, **90**, 4.

Reinking, O. A. & Manns, M. M. (1933). Parasitic and other *Fusaria* counted in tropical soils. *Z. Parasitenk.* **6**, 23.

—— —— (1934). Parasitic and other *Fusaria* counted in Columbia soils. *Zbl. Bakt.* Abt. 2, **89**, 502.

RUSSELL, R. C. (1934). Studies of take-all and its causal organism, *Ophiobolus graminis* Sacc. *Bull. Dep. Agric. Can.* no. 170.

SADASIVAN, T. S. (1939). Succession of fungi decomposing wheat straw in different soils with special reference to *Fusarium culmorum*. *Ann. appl. Biol.* **26**, 497.

SAMUEL, G. & GREANEY, F. J. (1937). Some observations on the occurrence of *Fusarium culmorum* on wheat. *Trans. Brit. mycol. Soc.* **21**, 114.

SANFORD, G. B. (1939). Research on certain soil-borne diseases as affected by other micro-organisms. *Sci. Agric.* **19**, 609.

SCHAFFNIT, E. (1930). Ertragseinbüssen im Getreidebau durch Fusskrankheiten. *Mitt. dtsch. LandwGes.* **45**, 247.

SHEN, C. I. (1940). Soil conditions and the *Fusarium culmorum* seedling blight of wheat. *Ann. appl. Biol.* **27**, 323.

SIMMONDS, P. M. (1930). A washing device for isolation work with plant material. *Phytopathology*, **20**, 911.

TYNER, L. E. (1940). The effect of crop debris on the pathogenicity of cereal root rotting fungi. *Canad. J. Res.* Sect. C, **18**, 289.

(*Received* 10 *June* 1941)

EXPERIMENTS ON THE CONTROL OF CLUB ROOT OF BRASSICAE IN GARDENS AND ALLOTMENTS

A SUMMARY OF SIX YEARS' TRIALS

By N. C. PRESTON

Advisory Mycologist, Harper Adams Agricultural College, Salop

PRESTON (1928, 1931, 1934) showed that club root of brassicae can be controlled effectively on small areas by treating the dibble holes with a 0·05 % solution of mercuric chloride at planting time. Though this method of treatment has proved both practical and economic the poisonous nature of the fungicide has undoubtedly militated against its very general use, partly owing to the supposed risk involved and partly because many small growers and allotment holders, to whom such a simple treatment should be of particular value, have found difficulty in obtaining mercuric chloride from their retail chemists. During the past six years, therefore, the action of a variety of other substances upon club root in the field has been investigated in the hope that some of these might prove useful substitutes for mercuric chloride while being without its disadvantages. Many of the materials tested proved useless, but with three of them the measure of control obtained was of practical significance and the experiments with these are therefore described in some detail. Reference is also made to experiments with some of the less effective substances which appear likely to be of interest in spite of the negative or inconclusive results obtained with them.

GENERAL PROCEDURE

With certain exceptions, which are referred to specifically, the experiments were conducted on a heavily and uniformly infected plot of land at the Harper Adams Agricultural College. The plot was approximately 40 × 6 yd. and the plants were set across it in rows of twelve, 18 in. being allowed between the plants in each row and the rows spaced 2 ft. apart. The manurial treatment consisted of light dressings of farmyard manure only, no artificial manures being applied.

The substances under test in any one season were applied either directly to each of the dibble holes in the rows allotted to them, or were previously distributed in shallow drills corresponding to the rows in which the plants were afterwards set. The various treatments, including controls, were randomized in blocks in which a single row, or more frequently two adjacent rows, were allotted to each treatment. A separate randomization was made for each unit block and, with a single exception, a minimum of five repetitions of these units was made.

Recording of results. Cabbages or cauliflowers, and in a single instance savoys, were used as test plants. These were harvested when mature and the number and weight of the marketable and unmarketable heads was recorded. After cutting, the roots were lifted and graded, according to extent and type of clubbing present, as follows:

(1) Severe: tap root, and laterals if still recognizable, heavily clubbed.
(2) Moderate: clubbing of the finger type wholly or chiefly confined to the lateral roots.
(3) Slight: swellings few or small, on laterals only.
(4) Free: no clubbing visible.
(5) Indeterminate: roots too decayed to allow assessment of clubbing; usually the result of root fly attack and/or very severe early infection with club root.
(6) Plants lost: no trace remaining at time of lifting, cause of disappearance doubtful.

Substances tested and results obtained

Calomel. In previous experiments (Preston, 1928, 1931) calomel had been tested by dusting the chemical directly on the roots of the seedlings before planting. Under these conditions it proved unsatisfactory and was temporarily discarded in favour of mercuric chloride. In these trials it was applied to the soil, just before planting, either in the dibble holes or along shallow drills. It was used alone or mixed by hand with dry sifted soil or, as in the 1935 and 1936 trials, was obtained ready mixed with 85% aluminium silicate as a filler. In 1935 and 1937 tests were carried out in school gardens in north Staffordshire where, owing to the size of the plots available, the number of plants per row varied from ten to twenty-five. The treated and control rows were here arranged alternately, the normal 2 ft. being allowed between each. Besides these tests experiments with calomel were conducted upon the College plot, from 1936 to 1940 inclusive. Quantities varying from 0·08 to 0·6 g. pure HgCl per plant were used. The results obtained from this series of experiments are shown in Table 1.

Table 1. *Effect of calomel applied to the soil at various rates before planting*

Year	Rate of application g. per plant	No. planted	No. lifted	Av. wt. per head	Market heads %	Severe %	Mod. %	Slight or absent %	Indeterminate, etc. %	Plants lost %
1935	0·08	63	56	0·9	39·7	57·1	4·8	15·9	11·1	11·1
	0·2	54	40	—	31·5	38·9	7·4	24·1	5·6	24·0
	0·6	36	29	0·6	19·4	11·1	2·8	50·0	16·7	19·4
	Nil	162	153	0·5	12·0	77·8	2·4	2·5	5·6	11·7
1936	0·25	108	102	—	52·8	5·6	5·6	75·5	8·9	4·4
	Nil	108	89	—	23·1	56·7	3·4	12·1	8·9	18·9
1937:										
(*a*) College	0·5	120	120	2·6	63·0	19·0	7·0	65·0	9·0	—
	Nil	100	100	1·4	25·0	58·0	3·0	14·0	25·0	—
(*b*) School	0·3	120	113	—	12·5	57·5	5·8	10·0	12·5	0·8
	Nil	120	119	—	12·7	76·7	9·2	8·3	5·0	1·7
1938	0·5	50	46	—	44·0	12·0	12·0	52·0	16·0	8·0
	Nil	70	65	—	6·0	60·0	4·3	8·6	20·0	7·1
1939	0·125	60	57	0·8	15·8	31·7	8·4	18·3	41·6	—
	0·25	60	59	1·0	13·6	40·0	20·0	26·7	13·3	—
	0·5	60	60	1·7	62·0	26·7	25·0	31·7	16·6	—
	Nil	96	90	0·3	1·0	44·0	0·0	0·0	56·0	—
1940	0·5	60	59	1·0	41·7	31·7	35·0	25·0	8·3	—
	Nil	120	117	0·3	5·8	70·0	0·8	1·7	25·0	2·5

Mercury-zinc amalgam

According to Daines (1936) the fungicidal value of mercury compounds depends upon their reduction in the soil to metallic mercury, the vapour from which becomes the toxic agent. He found, moreover, that metallic mercury in the presence of certain other metals was more toxic to *Rhizoctonia* than were the mercurials previously used to control this fungus. It appeared worth while, therefore, to find out whether similar results could be obtained by using metallic mercury against club root, especially as mercuric chloride had proved so satisfactory in controlling this particular disease.

The amalgam used consisted of 12% metallic mercury and 6% zinc incorporated with

82% calcium carbonate as filler,* the mixture being in the form of a fine powder. It was first tested on a few plants in 1936 and more adequately in 1938 and 1940. The material was applied to the soil at the rate of 48 g./15 ft. drill length in the 1938 trials, and directly to the dibble holes in 1940 when the rates of application were 4 g. and 8 g. (=0·5 and 1·0 g. Hg) per plant respectively. The results of these trials are shown in Table 2.

TABLE 2. *Effect of mercury-zinc amalgam applied to dibble holes before planting*

Year	Treatment Hg equiv. g. per plants	Plants set no.	Plants cut no.	Marketable heads %	Clubbing of roots: Severe %	Moderate or finger %	Slight or absent %	Indeterminate %	Missing %
1938	0·5	50	39	30·0	6·0	12·0	54·0	6·0	22·0
	Nil	70	65	6·0	60·0	4·3	8·6	20·0	7·1
1940	0·5	120	120	56·7 (av. wt. 1·1 lb.)	30·0	43·3	23·3	3·4	0·0
	1·0	120	120	38·3 (av. wt. 0·9 lb.)	43·3	39·2	10·0	7·5 =	0·0
	Nil	60	58	1·7 (av. wt. 0·3 lb.)	70·0	0·0	3·3	23·3	3·4

An additional series of plots was arranged on badly infected farm land at Newport, Salop, where the amalgam, instead of being applied to the individual drills or plants, was broadcast over the soil surface and lightly raked in just before planting. This series consisted of seven plots occupying a strip of land 14 × 3 yd., the plots which thus measured 3 × 2 yd. were arranged as shown in the diagram. Plots 1, 3 and 5 received 66 g. of the mercury-zinc

amalgam mixture (=20 lb. metallic amalgam/acre); plot 7 received double this quantity, viz. 132 g. (140 lb. metallic amalgam/acre) and plots 2, 4, 6 were left untreated. Twenty-five cabbage seedlings were planted out on each plot and the crop was harvested and the roots examined on 1 Oct. 1940. An interim examination of the growing crop was made on 26 Aug. the plants being then graded by inspection with the following results:

	Healthy plants: Large or medium	Healthy plants: Small	Diseased plants: Large or medium	Diseased plants: Small	Missing
Amalgam % (all four plots)	53	3	11	19	12
Untreated %	9·3	4	16	52	18·7

The figures obtained in the final count made on 1 Oct. are shown in detail in Table 3.

* Experiments with calcium carbonate alone indicated that the use of this substance as a filler did no materially affect the results.

TABLE 3. *Effect of mercury-zinc amalgam broadcast immediately before planting*

	No. lifted	Marketable %	Total weight	Severe %	Moderate %	Slight or absent %	Indeterminate %	Missing %
Hg-Zn amalgam: 66 g. per plot	66	38	102	48·0	5·3	26·7	8·0	9·0
Hg-Zn amalgam: 132 g. per plot	23	52	29	28·0	4·0	56·0	4·0	8·0
Untreated	62	7	34	68·0	0·0	4·0	17·3	20·7

Brassisan

Trials with this substance were carried out in 1936 and 1937 on the College plot, the plants being set out in randomized rows as previously described. The material was applied to shallow drills before planting and in each of the test years it was used at approximately the same rate, viz. 50 g./14 ft. drill length. As shown in Table 4 the effects of this treatment were generally comparable with those obtained with calomel in the same season.

TABLE 4. *Effect of brassisan upon club root when applied to drills before planting. Rate of application 50 g./15 ft. drill length*

Year	Crop	Treatment	Plants set	Marketable %	Av. wt. per head lb.	Blind %	Extent of clubbing: Severe %	Moderate %	Slight or absent %	Indeterminate %	Missing %
1936	Cauliflowers	Brassisan	108	19·4	—	20·5	10·2	3·7	42·6	27·8	15·7
		Nil = Controls	108	23·1	—	16·7	56·7	4·4	11·1	8·9	18·9
1937	Cabbage	Brassisan	100	65·0	3·0	—	22·0	8·0	59·0	11·0	0·0
		Nil = Controls	100	25·0	2·8	—	58·0	3·0	14·0	25·0	0·0

Miscellaneous substances

Under this heading are grouped trials carried out over the six-year period with a variety of chemicals which, for one reason or another, it was thought might prove of value but which failed to give a satisfactory control of club root under the conditions of experiment. It is possible, however, that under different conditions and methods of application some of these materials might be useful and worth further investigation.

(*a*) *Indine blue* (= *New Blue R*) *and Auramine.* These dyes are apparently very toxic to bacteria at extremely high dilutions (Fairbrother & Renshaw, 1923). They were used as dry powders, 25 g. of the dye being incorporated with 250 g. dry hydrated lime and applied at the rate of 50 and 100 g. of the mixture/15 ft. drill length. Other drills were treated with hydrated lime only at rates of 50 and 100 g./drill, to serve as additional controls. The dyes themselves apparently had no effect upon infection of the plants but in both the drills treated with the dye-lime mixture and in those receiving lime alone clubbing was reduced to 9% from the 20% recorded for the *untreated* controls.

(*b*) *Malachite green.* This dye when tested some years previously (Preston, 1928) in the form of 0·01 and 0·005% solutions gave no appreciable control of club root. In 1936, however, it was incorporated with aluminium sulphate as a filler and used dry. This mixture, containing 5% malachite green, was applied at the rate of 20 g./15 ft. row, = approx. 0·1 g. of the dye per plant. Severe clubbing was reduced from 61% on the control rows to 36·6% on the treated ones but there was no corresponding increase in the number of marketable heads produced, and no appreciable control of cabbage root fly was observed.

(*c*) *Allyl compounds.* Rocklin (1933) showed that immunity to club root where it occurs among cruciferous plants is due to the presence of certain glucosides which, acted upon by enzymes, give rise to volatile aromatic mustard oils. An attempt was made to discover whether any reduction of clubbing could be obtained by the direct application of such substances to the plants or to the soil.

The compounds selected were allyl isothiocyanate and allyl sulphide and two methods of application were adopted:

(1) The roots of cabbage seedlings were immersed for periods of 3 to 46 hr. in 0·05 and 0·0125% solutions of allyl isothiocyanate before planting.

(2) Mustard seedlings growing in pots of infected soil were watered at intervals with similar concentrations of each of the two compounds used separately.

No significant reduction of clubbing was secured by either method and even the plants which had been allowed to absorb the solutions for 3 hr. only were adversely affected. Soon after planting the growing points became brown and within a few days most of these plants were dead.

Rocklin (1933) also described beneficial results obtained by treating soil and seedlings with an aqueous extract of black mustard (*Brassica nigra*), a species resistant to club root and known to contain the protective glucosides. In the following year (1936) therefore additional trials were carried out using ground-up seeds of *B. nigra* which were applied to the soil in drills before planting at the rate of 100 g./15 ft. drill length. The results were negative, no significant difference between treated and untreated rows being obtained.

Magnesium sulphate. On repeated occasions the writer has been told by gardeners that this compound is very effective against club root and it was decided to test it experimentally. This was done in 1934 and 1937. Care was taken to apply the chemical according to the instructions of those advocating it and it was therefore used as a 1·25% solution (1 lb./8 gal.) and applied at the rate of ½ pint per plant. No beneficial effect whatever was obtained. Not only did the treatment fail to reduce clubbing but the treated plants cropped rather less satisfactorily than the corresponding controls.

(*d*) *Mercuric chloride.* The efficacy of this chemical in controlling club root has been clearly demonstrated in previous trials (Preston, 1928, 1931, 1934). In the present series of experiments it was employed in 1937, 1938 and 1940 as a standard of comparison and was used either as a 1/2000 or 1/1500 solution applied at the rate of ½ pint per plant. The results were generally superior to those given by any of the substances tested at the same time. In 1937, however, a slightly higher reduction of severe clubbing was obtained with the Hg-Zn amalgam, the figures being:

Controls	60%	severe	clubbing
Mercury zinc	6%	,,	,,
Mercuric chloride	12%	,,	,,

Discussion of results

It is evident from the foregoing tables that none of the substances used gave complete immunity from club root infection. It is equally clear that the mere presence or absence of clubbing in the general sense is no criterion of the practical success or failure of the treatment employed. What is of obvious importance is how far the disease affects the cropping capacity of the plants and this appears to be determined by the type or nature of the clubbing produced. In earlier experiments the writer tended to group together the badly and moderately clubbed roots and to compare their number with that of those slightly affected or clean. The experience of the last five years, however, shows that this is not an altogether correct way of judging the results. It is rather the percentage of the 'severely clubbed' roots alone which is chiefly reflected in the reduction of marketable heads and weight of crop obtained. From purely practical considerations clubbing of the moderate or finger type is much less detrimental and in computing results such infections should therefore be grouped in the 'slight' rather than in the 'severe' category. This was particularly evident in the 1940 trials. As the plot received no fertilizer apart from a light dressing of farmyard manure crop weights were generally low, but whereas the untreated plants gave an average head weight of only 5 oz. the treated ones gave 1–1½ lb. At the same time a comparatively high percentage of roots heavily infected with the finger type of clubbing was observed among treated plants while among the untreated this was replaced

by very severe infections of the club type in which the entire root system was converted into a single solid mass.

Calomel. Table 1 shows that a substantial reduction of severe clubbing was obtained by the use of calomel each year. The degree of control, measured by this reduction, varied somewhat from year to year and, as might be expected, it was consistently greater when the larger quantities of calomel (0·5–0·6 g.) were used than for smaller amounts. In 1939 a reduction of only 17·3 % in severe clubbing was recorded. A possible explanation of this apparent failure appears, however, when the percentage of plants recorded as 'indeterminate' is considered combined with the clubbing figure. For convenience the percentage differences are tabulated here for quantities of calomel from 0·5 to 0·6 g. When the 'indeterminate' figure is added to the club reduction figure a remarkably consistent result is obtained.

	A Reduction of severe clubbing %	B Reduction of 'indeterminate' %	A+B
1935	66·7	−11·1	55·6
1936	—	—	—
1937	39·0	+16·0	55·0
1938	48·0	+ 4·0	52·0
1939	17·3	+39·4	56·7
1940	38·3	+14·7	53·0

The 'indeterminate' roots were those so destroyed that the degree of clubbing could not be estimated. It can safely be assumed that in the great majority, and quite probably in all of them, the destruction was brought about either by severe clubbing at an early stage or by root fly attack or by a combination of these two factors. While, therefore, this figure may be considered to indicate the incidence of root fly the fact that many such roots were probably also infected with club root, or might well have become so had the plants survived, cannot be ignored. The combined beneficial effect of calomel in simultaneously reducing both club root and root fly infection is thus apparent throughout the six-year period.

Mercury-zinc amalgam. As Table 2 shows, the results obtained when 0·5 g. of mercury in the form of this amalgam was applied to the dibble holes, are closely comparable with those obtained by using 0·5 g. calomel in the same season, both as regards the reduction of severe clubbing and the reduction of 'indeterminate' roots, i.e. control of root fly, etc. This effect is reflected also in a substantial increase of marketable heads and of crop weight, in spite of the relatively high percentage of 'moderate' clubbing recorded for the treated plants in the 1940 trials. This is in accordance with the view expressed previously that it is the severe clubbed type of root malformation rather than the finger type which occasions the material loss of crop. As with the calomel results there is also close agreement between the 1938 and 1940 figures when the severe clubbing and indeterminate percentages are considered in relation to each other. A somewhat unexpected result is that given by the 1 g. application. This doubling of the amount of amalgam applied to each plant resulted not only in a lower crop weight than that given by the smaller quantity but also in an actual increase in the percentage of severely clubbed and indeterminate roots. During the growing period there was a visible difference between the plants receiving quantities of the amalgam equivalent to 0·5 and 1·0 g. mercury respectively, the appearance of the latter being obviously less healthy throughout the season.

As regards the effects of the amalgam when applied broadcast, the number of plants and

plots involved is too small to warrant any definite conclusions, though the differences seem sufficiently great to suggest that a practicable control of the disease might be obtained by this means. At the rate of 40 lb. metallic amalgam/acre (or 2 cwt. of the mixture), however, the cost of such treatment, even based on a pre-war price of 2*s*. per lb., would be too high to be economic under market garden conditions. Smaller quantities of this amalgam would probably be inadequate but the question is worth fuller investigation.

Brassisan. As shown in Table 4 the results obtained in 1936 were quite comparable to those given by calomel as regards the reduction of clubbing and increase of marketable heads produced. In 1937 also the proportion of indeterminate roots was reduced by 23 % (calomel 22 %), but this improvement did not appear in the 1936 trials when 43·5 % indeterminate or missing roots were recorded, the figures for the calomel treatment and control plants in the same year being 13·3 % and 27·8 % respectively. In both these years there was only a moderate root fly attack and the close approximation of the control 'indeterminate' figures, 27·8 % in 1936 and 25 % in 1937, will be noted.

Conclusions from statistical analysis of 1940 *trials*

The results of the 1940 trials, which are summarized together below, were subjected to statistical analysis by the writer's colleague, Mr F. J. Dudley.

The trial plot in this year was arranged according to the usual plan previously described, the plants being set in randomized rows of twelve arranged as follows:

Rows 1–8	C_1	A	S_1	B	C_2	B	S_2	A
9–16	S_1	C_2	B	A	S_2	C_1	A	B
17–24	B	S_1	A	C_1	A	B	C_2	S_2
25–32	A	S_2	B	A	S_1	C_1	B	C_2
33–40	B	C_2	A	C_1	S_1	A	S_2	B

where A = mercury-zinc amalgam 0·5 g. Hg per plant,
B = mercury-zinc amalgam 1·0 g. Hg per plant,
S = 'standard' treatments: S_1 calomel 0·5 g. per plant,
S_2 mercuric chloride, 1/1500 solution,
C = 'controls': C_1 calcium carbonate 1 g. per plant,
C_2 no treatment.

Each treatment is thus repeated ten times, the two standards being considered as one 'treatment' and likewise the two sets of controls since the main object of this experiment was to test the mercury-zinc amalgam against the two already proved standard treatments.

The effects of the treatments were considered from various aspects and the conclusions arrived at by these separate analyses are summarized here:

(1) *Effect on incidence of disease.*

(*a*) *Severe clubbing only.* The control treatments C_1 and C_2 were almost identical in effect and the incidence of severe clubbing was significantly higher than that given by any of the other treatments. There is a significant difference between the figure given by the standard treatment S ($HgCl_2$ and Hg_2Cl_2 together) and those of treatment B (Hg-Zn amalgam 1 g. Hg equivalent). S_2 ($HgCl_2$) was significantly more effective than S_1 (calomel).

(*b*) *Moderate clubbing* (*finger type*) *only.* As regards the control of this type of clubbing the three main treatments A, B and S were not significantly different from one another nor was S_2 significantly different from S_1 in this respect. The effect of these treatments as compared with C (controls) appears to be a reduction in the extent of club root damage and at the same time a manifestation of the incomplete control of the disease.

(*c*) *Severe and moderate* (*finger type*) *damage.* When the two types of clubbing are considered together it is found that there is no significant difference between C, A, B and S while S_2 shows a significantly lower figure than the rest. Thus S_2 ($HgCl_2$) appears effective in controlling both types of damage and in this respect seems to be more successful than S_1 (calomel).

(*d*) *Assessment of value by scoring.* When a score was assigned to each plant according to the type of clubbing observed, viz. 1 for slight or no damage, 2 for indeterminate, 3 for moderate and 4 for severe clubbing, it was found on analysis that S_2 ($HgCl_2$) was a significantly better treatment than any of the others. S_1 (calomel) and A (Hg-Zn, 0·5 g. Hg) did not differ appreciably but each was significantly better than B, C_1 and C_2. There was no significant difference between B (Hg-Zn, 1 g. Hg) and C (controls).

(2) *Effect on crop.*

As far as the *number* of marketable heads produced is concerned the effects of A, B and S were significantly different in different blocks and in consequence no significant difference could be ascribed to these main treatments. S_2, however, differed from A and B. The figure given by C (controls) was so small that it was ignored in this analysis, being clearly significantly below that of each of the main treatments. Where *weight* of crop is concerned C_1 and C_2 are significantly inferior to the main treatments A, B and S. These, however, cannot be regarded as significantly different in effect from one another and the apparent superiority of S_2 over S_1 is here of doubtful significance.

From these observations it appears that mercuric chloride is clearly the most effective of all the substances tested in this particular series of experiments. At the same time the analysis shows that a significant control of club root has also been attained both by calomel and by the mercury-zinc amalgam at the lower rate of application. These two materials are of approximately equal efficiency in this respect.

General conclusions

The results of the trials carried out over the six-year period show that a useful and practical, though incomplete, control of club root can be obtained by applying to the soil any of the three substances: calomel, mercury-zinc amalgam, and brassisan. In general, however, none of these is superior to mercuric chloride used in the form of a 1/1500 solution, though all of them possess the practical advantages of being relatively non-poisonous and easily applied. Calomel is more easily obtained than mercuric chloride and its efficacy against cabbage root fly, even when applied beneath the soil, adds to its usefulness. Such considerations render these substances especially convenient to the allotment holder and gardener and may outweigh the question of cost, especially where comparatively few plants have to be treated. The relative costs of the three mercury treatments ($HgCl_2$, Hg_2Cl_2 and Hg-Zn amalgam) are approximately in the proportion of 1, $2\frac{1}{2}$ and 6 respectively.

Summary

Experiments with ten different substances in relation to the control of club root of brassicae are described. The experiments cover a period of six years and a statistical analysis of the last year's trials is included. Significant practical control of the disease was attained with calomel, a mercury-zinc amalgam and also with the proprietary brassisan. It is considered that these substances have certain advantages over mercuric chloride which may especially commend them for use in gardens and allotments. Mercuric chloride when used as a standard of comparison gave consistently better results than the other materials tested.

The writer gratefully acknowledges the practical co-operation of the headmasters of the Staffordshire Church and County Schools at Leek and Kidsgrove, Messrs H. C. Burdett and F. Bryan, and their staffs, who put land and practical help at his disposal, and of Mr J. R. Booer (Messrs Berk and Co., Ltd.), who suggested and provided the material for the tests with the mercury amalgam. His thanks are also due to Mr F. J. Dudley for his statistical contribution to this paper and to Mr W. C. Moore for helpful criticism and suggestions regarding the presentation of the text.

REFERENCES

DAINES, R. H. (1936). Some principles underlying the fungicidal action of mercury in soils. *Phytopathology*, **26**, 90.

FAIRBROTHER & RENSHAW (1923). The comparative toxicity of dyestuffs. *J. roy. Soc. Arts*, pp. 3668–9 (March).

PRESTON, N. C. (1928). Experiments on the control of finger and toe in cabbages by the use of mercuric chloride and other substances. *Welsh J. Agric.* **4**, 280–95.

—— (1931). The prevention of finger and toe (club root) in gardens and allotments. *J. Minist. Agric.* **38**, 272–84.

—— (1934). The control of club root (finger and toe) in cauliflowers. *J. Minist. Agric.* **41**, 329–35.

ROCKLIN, E[MILIA] (1933). Zur frage der widerstandsfähigkeit der cruciferen gegen die kohlhernie. *Phytopath. Z.* **4**, 381–406.

(*Received* 30 *May* 1941)

VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS

By B. KASSANIS, Ph.D. and F. M. L. SHEFFIELD, D.Sc.
Rothamsted Experimental Station, Harpenden, Herts

(With Plates 13 and 14)

Intracellular inclusion bodies have been found in only about twenty of the hundred or so plant virus diseases which have been described in detail, but in these they have often proved a useful aid in diagnosis and differentiation. If a virus induces them in one host in which it causes mosaic symptoms, it usually induces their formation in all those hosts which show such symptoms. They do not usually occur in plants showing necrotic symptoms or in carriers. The inclusions of different viruses often differ in morphology and show slight differences in physical and chemical properties, but those induced by any one virus have been fairly constant in form, subject only to slight modification by the host plant and to variation in quantity and distribution. However, in the summer of 1940, several new types of cytoplasmic inclusions were found in plants infected with each of three strains of tobacco mosaic virus: the ordinary tobacco mosaic (Johnson No. 1), aucuba mosaic and enation mosaic viruses.

In 1903, Iwanowski first described the inclusions in tobacco plants with mosaic. He described the amoeba-like bodies, of 5–10 μ diameter, which Goldstein (1926) later called *X*-bodies, and also the large plate-like crystals which become striated on treatment with dilute acids. Over a period of nearly 40 years this virus has been worked with, and its effects described by, many workers under varying conditions in several parts of the world. The phenomena observed have been interpreted in various ways but surprisingly few discrepancies exist in the accounts. Casual reference has sometimes been made to the occurrence of a few raphides in infected plants but otherwise descriptions have differed from Iwanowski's only in giving greater detail.

Enation mosaic virus has been less extensively studied. The intracellular inclusions when first examined at Rothamsted in 1936 were indistinguishable from those of tobacco mosaic virus, consisting of an amorphous protein *X*-body and of larger hexagonal protein crystals which were often fused side by side (Sheffield, 1939*b*; Bawden & Sheffield, 1939). This strain was carried on continuously through a series of hosts. Plants examined from time to time showed no variation until the summer of 1940.

Aucuba mosaic virus had shown some slight variation before 1940. It differed from the other two strains in that instead of the amoeboid body, it produced large inclusions of sometimes 30 μ diameter by the aggregation and fusion of particles of amorphous protein material, which were precipitated in the streaming cytoplasm. After some weeks crystals were formed within this body. Under some conditions, the amorphous body was not formed but crystals were formed directly. With the amorphous body was often found a long spike-like inclusion (Henderson Smith, 1930; Sheffield, 1931). This inclusion was present

in 1928 and occurred regularly in 1929 and 1930. For four years no records were made but in 1935 its disappearance was commented on and from that time, although it was frequently sought, it was not again observed. The appearance of such spike-like bodies in very large numbers in a plant infected with tobacco mosaic virus led to the discovery of a large number of variations in the cytology of plants infected with any one of the three strains. This paper describes and discusses the new forms.

Material

When, early in September 1940, the plant suffering from tobacco mosaic was found not to be behaving typically, all other plants in the glasshouses infected with any of the three strains of virus were immediately examined. These, also, all contained atypical inclusions. Inoculations were then made to seedlings of tobacco, tomato and *Solanum nodiflorum* from these plants and also from dried, or purified, specimens of virus. The tobacco mosaic virus used came originally from Dr James Johnson, some had been carried on at Cambridge and some at Rothamsted for many years. Samples of both had been dried or purified at various times over a period of years. The aucuba mosaic virus came originally from Dr W. F. Bewley and had been maintained in the Rothamsted glasshouses for 15 years. Specimens had been dried at a time when spikes were known to be present and also dried or purified when they were known to be absent. The enation mosaic virus was obtained from Dr G. C. Ainsworth in 1936 and has since been carried on in the Rothamsted glasshouses. Some of the inocula were kindly supplied by Mr F. C. Bawden and by Dr J. Henderson Smith. In all, over a dozen sources of virus were used. One or more sets of inoculations were made from each, a minimum of three plants being used for each inoculation. As the results obtained appeared to be independent of the sources of the inocula these are not given in detail.

Most plants were kept in the glasshouse chambers, no attempt being made to control the amount of light or heat available. Early in October a few plants were kept shaded. Tobaccos were infected with tobacco mosaic virus and tomatoes with aucuba mosaic virus. Some of each group put in slight shade became etiolated, and some in dense shade became very stunted.

Variations

Crystalline inclusions

Hexagonal crystals (Purdy Beale, 1937; Bawden & Sheffield, 1939) were produced by all three strains in all hosts and were found at some period in every plant examined. In tobacco and enation mosaics they appeared soon after the external symptoms. In the plants inoculated in May and June with aucuba mosaic virus their appearance was long delayed as amorphous bodies were first formed and crystals were derived only from these (Pl. 14, fig. 1*a–c*). In plants inoculated on 19 June and 4 Sept. the bodies began to crystallize in November. Plants inoculated with aucuba mosaic virus in October produced no amorphous bodies but crystals were present in November although no external symptoms were apparent. When plants were shaded from the time of inoculation with aucuba mosaic virus no amorphous bodies were formed but crystalline material appeared after about 7 weeks. Shaded plants inoculated with tobacco mosaic virus produced both amoeboid bodies and hexagonal crystals but not until 7 weeks after infection. The hexagonal crystals are striate in edge view but this is usually seen only through crossed Nicol prisms or on acidification. Occasionally the striations are very conspicuous even in crystals in untreated cells (Pl. 13, fig. 5). This occurs usually in cells which in summer produced the more usual types of inclusions and in winter were forming rather unusual amorphous inclusions.

Fibrous inclusions

Spike-like bodies. These fine needle-shaped bodies had occurred regularly in plants infected with aucuba mosaic virus but had disappeared for some years. Raphides which have occasionally been mentioned in descriptions of tobacco mosaic disease were probably similar; these had never been found at Rothamsted.

Early in September 1940 spike-like bodies were found in all the hosts which had been inoculated in May, June and July with any of the three virus strains. In *S. nodiflorum* infected with aucuba mosaic virus they were about as abundant as in 1929, usually one, occasionally more, occurring in

most of the hair cells and in every cell over large areas of the epidermis. In tobacco infected with tobacco mosaic virus they were very abundant, several being present in almost every epidermal cell (Pl. 13, fig. 1). They appeared first in the hairs and in the epidermis a few days later. On 9 Sept. inoculations were made of all three strains, each obtained from several sources, to a large batch of tobacco plants. Spike-like bodies appeared in all those plants which developed systemic symptoms. Some of these plants were destroyed on 21 Oct.: others kept until the end of November ceased to produce this form of inclusion, which could then be found only in the older leaves. A few were found in *S. nodiflorum* inoculated on 19 Sept. with aucuba mosaic virus but otherwise none was found in plants inoculated later than 9 Sept. although they were present in all plants inoculated between May and that date. In November they were found to be disappearing even from the older leaves of plants in which they had been abundant.

These inclusions were fine needle-shaped bodies with pointed ends and no facets. In length they are usually approximately equal to the longest dimension of the containing cell. Henderson Smith (1930) said that the spike 'can sometimes be seen to be made up of a bunch of hair-like crystals, especially distinguishable at the ends'. Sheffield, who has worked with this virus since 1929, failed to observe this condition until these bodies reappeared in 1940. Careful examination between crossed Nicol prisms often revealed that the body consisted of a number of extremely delicate fibres lying parallel or twisted together. Slight acidification caused these fibres to separate and to become easily visible by transmitted light. Further acidification caused their dissolution. They were weakly birefringent (Pl. 13, fig. 8), of the same sign as the cell wall, the refractive index being higher in the direction of the length of the fibre. They could be fixed in formol-saline or in saturated aqueous picric acid.

Spindle-shaped bodies. Usually only one spindle-shaped body was found in a cell (Pl. 13, fig. 7). It might be about the length of the cell or, if in a very long hair cell, might lie diagonally across one half of it. They were 10–15 μ wide at the centre tapering to extremely fine ends which might be curved. They consisted of aggregates of long fine fibres and might well have been composed of a number of the spike-like inclusions of various lengths packed closely side by side. When viewed between crossed Nicol prisms they were doubly refractive and their fibrous structure became more obvious. They were induced by all three virus strains but were not found in *S. nodiflorum*. They were seen in plants infected in summer but not in those inoculated later than 9 Sept.

Masses of small needle-like fibres. With all three virus strains were found large numbers of fine needle-shaped bodies (Pl. 13, fig. 4). In shape, they resembled the spike-like bodies but were very much finer and shorter: usually their length was less than the width of a hair cell. When seen between crossed Nicol prisms they were weakly birefringent, the higher index being in the direction of the length. In size and appearance they were identical with the fibres formed by the acidification of the hexagonal crystals. Their position often suggested that they might be derived from these. The *p*H of sap extracted from cells containing them was about 5·6 which is insufficiently acid to produce needle-like forms experimentally from striate material. They also resembled the birefringent para-crystals produced by precipitating the virus with acid below *p*H 4 or by addition of large quantities of salts (Stanley, 1936; Bawden & Pirie, 1937). The *p*H of the cell sap is too high and the salt content insufficient to cause the separation of these para-crystals in the living cell. It is known that the masses of needle-shaped bodies were sometimes derived from amorphous bodies of the aucuba type (Pl. 14, fig. 2*a*) and also from a form of amorphous inclusion which will be dealt with later. Possibly they were also precipitated directly from the cell sap. Of this, it would be difficult to obtain conclusive evidence. They occurred most abundantly in summer but were found occasionally in winter in the older leaves of tomato plants inoculated in early summer. They were not found in *S. nodiflorum*.

Long curved fibres. Greatly elongated fibres were also found either alone in a cell or in association with any of the other forms of inclusion. Structurally they seemed to be similar to the spike-like forms but were several times as long as the cell, being curved and twisted within it. Often they were bent into the form of a figure 8 (Pl. 13, fig. 6). Some in long hair cells were calculated to be as much as 400 μ in length. Circles and tightly coiled fibres wound in the form of a sphere were occasionally found. Like other fibrous forms they were birefringent. They may be similar to the 'rings and figure 8's' described by Soukoff & Vovk (1938) as occurring in a mosaic disease of oats. They may be comparable to the mesomorphic fibres of as much as 2·5 mm. in length which separate from clarified sap from infected tobaccos standing at 1° C. for several months (Best, 1937). These fibres were produced by all three strains. They seemed to occur most abundantly in tomato and were never found in *S. nodiflorum*. They were most abundant in summer but still persisted in winter in some of the older leaves of tomato plants which had been inoculated in early summer.

Amorphous bodies

Several different types of amorphous inclusion occurred. The amoeboid type characteristic of tobacco and enation mosaics was found in all plants infected with these two viruses. It is difficult to make a quantitative estimate but the general impression was that they were seen much less frequently than in plants previously examined. These bodies had never been found in plants infected with aucuba mosaic virus until 1940 when similar structures occasionally appeared (Pl. 14, fig. 6).

The large granular amorphous bodies characteristic of aucuba mosaic were formed in all plants showing systemic infection and inoculated before the end of September. Plants inoculated after that date produced no amorphous inclusions but gave striate material directly. These amorphous bodies, if viewed between crossed Nicol prisms soon after formation, are not birefringent but after some weeks they come to contain a large amount of doubly refractive material (Pl. 14, fig. 1*a–c*): some of this usually takes the form of hexagonal crystals. In the summer of 1940, the bodies came to contain very large numbers of the small needle-like fibres often to the exclusion of all striate material (Pl. 14, fig. 2*a*). All stages could be seen between bodies containing only amorphous material and those which were almost completely translated to needle-like fibres. Some amorphous material is always left; presumably it contains the chondriosomes and other substances known to be present in the amorphous bodies. In summer one amorphous body might give rise to both striate material and fibres but towards autumn usually striate material only was formed. The contents of such bodies are in motion: sometimes it is so slow as to be visible only after several hours' observation but at others it is very rapid. In the latter case it usually occurs in loosely packed bodies containing small particles. Sometimes accompanying such disintegrating bodies of this type are found large numbers of smaller bodies bearing some resemblance to the amoeboid bodies of tobacco mosaic. They take the form of hyaline vesicles, usually spherical, and are not more than 10 μ in diameter. Each contains several highly refractive granules. These bodies either float in the cell sap or are carried rapidly in the cytoplasmic stream (Pl. 14, fig. 2*a–b*).

Until recently the only amorphous bodies which had been found in tobacco or enation mosaic infected plants had been of the amoeboid type. In December 1940 and the following weeks very large loosely packed masses of amorphous material were found (Pl. 14, figs. 3–5). This material was sometimes mixed with fibres. The fibres and some of the other particles were birefringent. The material in the masses was often closely packed but sometimes a hyaline vesicle with a few granules gave very much the appearance of an aucuba mosaic body immediately after pricking (Sheffield, 1939*a*). Also in the cell were often found large numbers of the small hyaline spherical bodies just described as occurring in aucuba mosaic. These usually contained highly refractive granules which were either spherical or rectangular. The contents of cells which contain these bodies are usually in such active movement that they are very difficult to photograph with an ordinary camera. The cytoplasm flowed rapidly carrying the smaller bodies with it. Particles within the large mass also moved. A small amount of striate material was sometimes found but more usually it was absent. In adjacent cells all the striate material was often found to have fused to a single mass. It is thought probable that these amorphous masses originate in striate material which has fused (as in Pl. 13, fig. 4) and undergone a change. Masses of this type were found in tobacco and tomato with all of the three strains of virus. They occurred most frequently in old tomato leaves especially in the cells of the large hairs of the petiole.

Discussion

The variations recorded * cannot be due to mutation of the virus strain for almost identical results were obtained from all three strains. Also inoculations were made from material preserved at different times over a period of 13 years. All inoculations made at the same time gave similar results. Further, when an infected plant was kept for some months the reaction of the later formed leaves was often different from that of the first formed leaves.

Inclusions may be modified slightly in form according to the host containing them (Sheffield, 1931). Further evidence is now afforded of the influence of the host plant. In *S. nodiflorum* far less variation was shown than in other hosts: the greatest variety was shown by tomato, especially in the largest hair cells. It is possible that the conditions which control the form taken by the intracellular inclusions also to some extent control their

* *Note added to proof.* Every type of intracellular inclusion body described in this paper was observed again during the early summer of 1941.

distribution throughout the tissues of the host plant. Hirayama & Yuasa (1937), in Japan, reported the regular occurrence of inclusions in the guard cells of the stomata of plants infected with tobacco mosaic virus, but Sheffield (1936*a*) was unable to find them and thought that the differences might be due either to the virus strain used or to the climatic conditions. The latter appears to be the more probable cause, for inclusions have now been found in the stomatal guard cells of one such plant grown at Rothamsted. Although they have frequently been found here in solanaceous plants infected with severe etch virus (Kassanis, 1939; Sheffield, 1941) where large numbers of inclusions are found in almost every tissue of the plant, it is the first time that inclusions have been found at Rothamsted in the guard cells of any plant infected with a tobacco virus strain although they have often been looked for. Even in 1940 they occurred rarely and were found only in September in one tobacco plant which was inoculated in July with tobacco mosaic virus. They have not been found in plants infected with the aucuba or enation strains.

Variations in the inclusions can be correlated to some extent with growing conditions and also with external symptoms which are themselves modified by growing conditions. In *Nicotiana glutinosa*, infection with any of these strains has always been confined to necrotic lesions around the points of entry of the virus and no intracellular inclusions are produced (Sheffield, 1936*b*). In tobacco, infection with aucuba mosaic virus may be localized when no inclusions are formed, or it may be systemic when inclusion bodies are many and various. With any of the strains, when external symptoms are very definite as in summer, inclusions are large, numerous and most varied. However, plants may show almost no external symptoms but still contain inclusions, which are then smaller, fewer and mostly of one kind.

The plants inoculated during the last seven months and examined during the last four months of 1940 made it obvious that the form taken by the inclusions varies with the season, all new fibrous forms were produced during the summer and the modified amorphous forms occurred principally in winter in plants inoculated in early summer. As winter approached all plants tended to form striate material to the exclusion of other forms. It seems probable that the chief factors determining the form of the inclusions are light and temperature. These virus strains multiply most rapidly in quickly growing plants and in winter artificial light was often supplied. It was found some years ago that plants infected with aucuba mosaic virus sometimes formed striate material but never produced amorphous inclusions in winter unless given additional light. This was provided by means of $\frac{1}{2}$ or 1 kW. electric bulbs with suitable reflectors over a period of 4–8 hr. during the night: such light was used every winter from 1930 to 1938. Although it was insufficient to produce the rapid growth usual in summer, amorphous inclusions were completely formed in about three weeks after inoculation. During May, June, August and September of 1940 the total hours of bright sunshine recorded at Rothamsted were well above the average for even that time of year (Table 1). It was during these months that all the plants showing exceptional forms of inclusion body were inoculated: unfortunately no plants were kept in shade during these months.

Comment has been made on the disappearance of the spike-like body for a period of years. It was present during the summers of 1928–30 and the amount of sunshine recorded was well above the average for the first two and just average for the third of these years. From 1931 to 1934 no records were made of the occurrence of the spike, so that it is possible that it disappeared and reappeared. In 1931–2, the hours of bright sunshine were well below

the average, whilst in 1933 they far exceeded it. In 1935 the absence of the spike was recorded and it was not seen again until 1940 although it was frequently looked for during the intervening years. During these years sunshine was poor. If sunshine be the sole determining factor it is strange that so many forms of inclusion were found in 1940 and far fewer in 1929 when the actual hours of light were greater than in 1940. It seems probable that temperature is a second important factor. In 1940, whilst receiving more than the usual amount of light, plants were getting less heat than in previous years. During the summer of 1940, no artificial heating was supplied to the glasshouses, whilst in the winter of 1940–1 they were heated only at night sufficiently to prevent damage from frost. Prior

TABLE 1. *Hours of bright sunshine recorded at Rothamsted**

Figures in italics show deviations from the averages which were taken over 48 years

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Totals
Av.	52·7	70·0	119·0	152·4	198·5	205·2	198·2	187·7	150·8	105·9	63·4	43·6	1547·4
1928	64·9	100·2	92·8	127·3	169·8	230·0	276·3	193·0	212·0	126·5	72·1	48·9	1713·8
	+12·2	*+30·2*	*−26·2*	*−25·1*	*−28·7*	*+24·8*	*+78·1*	*+ 5·3*	*+61·2*	*+20·6*	*+ 8·7*	*+ 5·3*	*+166·4*
1929	39·5	67·2	184·7	155·1	261·0	226·5	243·7	196·7	206·0	120·1	78·0	75·3	1853·8
	−13·2	*− 2·8*	*+65·7*	*+ 2·7*	*+62·5*	*+21·3*	*+45·5*	*+ 9·0*	*+55·2*	*+14·2*	*+14·6*	*+31·7*	*+306·4*
1930	48·8	59·1	123·5	114·8	166·3	242·6	194·6	226·0	125·0	134·9	76·6	31·2	1543·4
	− 3·9	*−10·9*	*+ 4·5*	*−37·6*	*−32·2*	*+37·4*	*− 3·6*	*+38·3*	*−25·8*	*+29·0*	*+13·2*	*−12·4*	*− 4·0*
1931	64·8	65·4	153·6	115·7	172·6	198·0	157·8	155·6	120·6	118·4	68·9	40·5	1431·9
	+12·1	*− 4·6*	*+34·6*	*−36·7*	*−25·9*	*− 7·2*	*−40·4*	*−32·1*	*−30·2*	*+12·5*	*+ 5·5*	*− 3·1*	*−115·5*
1932	50·5	67·6	144·2	131·3	128·4	215·5	136·3	191·5	113·2	104·1	47·4	56·2	1386·2
	− 2·2	*− 2·4*	*+25·2*	*−21·1*	*−70·1*	*+10·3*	*−61·9*	*+ 3·8*	*−37·6*	*− 1·8*	*−16·0*	*+12·6*	*−161·2*
1933	70·4	102·4	196·9	153·4	168·2	240·6	246·2	243·2	183·3	94·6	51·3	41·4	1791·9
	+17·7	*+32·4*	*+77·9*	*+ 1·0*	*−30·3*	*+35·4*	*+48·0*	*+55·5*	*+32·5*	*−11·3*	*−12·1*	*− 2·2*	*+244·5*
1934	56·9	96·1	127·0	120·8	200·8	184·9	274·8	180·4	172·6	85·0	45·9	20·9	1566·1
	+ 4·2	*+26·1*	*+ 8·0*	*−31·6*	*+ 2·3*	*−20·3*	*+76·6*	*− 7·3*	*+21·8*	*−20·9*	*−17·5*	*−22·7*	*+ 18·7*
1935	46·7	53·0	134·3	126·7	193·8	195·0	280·1	203·9	149·9	112·1	61·9	47·5	1604·9
	− 6·0	*−17·0*	*+15·3*	*−25·7*	*− 4·7*	*−10·2*	*+81·9*	*+16·2*	*− 0·9*	*+ 6·2*	*− 1·5*	*+ 3·9*	*+ 57·5*
1936	49·6	81·0	86·0	126·8	177·0	182·8	120·9	181·2	84·4	97·1	46·3	59·7	1292·8
	− 3·1	*+11·0*	*+33·0*	*−25·6*	*−21·5*	*−22·4*	*−77·3*	*− 6·5*	*−66·4*	*− 8·8*	*−17·1*	*+16·1*	*−254·6*
1937	44·4	64·4	104·5	95·3	158·3	187·6	126·1	187·1	138·7	78·3	69·3	24·1	1278·1
	− 8·3	*− 5·6*	*−14·5*	*−57·1*	*−40·2*	*−17·6*	*+27·9*	*− 0·6*	*−12·1*	*−27·6*	*+ 5·9*	*−19·5*	*−269·3*
1938	47·1	67·0	176·6	157·1	161·4	203·1	143·4	151·1	120·0	117·7	68·9	45·4	1458·8
	− 5·6	*− 3·0*	*+57·6*	*+ 4·7*	*−37·1*	*− 2·1*	*−54·8*	*−36·6*	*−30·8*	*+11·8*	*+ 5·5*	*+ 1·8*	*− 88·6*
1939	45·7	106·0	95·6	164·8	159·1	205·2	157·5	151·7	142·0	90·0	38·0	44·2	1399·8
	− 7·0	*+36·0*	*+23·4*	*+12·4*	*−39·4*		*−40·7*	*−36·0*	*− 8·8*	*−15·9*	*−25·4*	*+ 0·6*	*−147·6*
1940	86·7	22·5	127·1	124·0	224·8	267·9	189·8	191·1	170·9	93·9	76·6	41·2	1616·5
	+34·0	*−47·5*	*+ 8·1*	*−28·4*	*+26·3*	*+62·7*	*− 8·4*	*+ 3·4*	*+20·1*	*−12·0*	*+13·2*	*− 2·4*	*+ 69·1*

* This table was adapted from data published in the *Annual Reports of the Rothamsted Experimental Station.*

to 1940, heat was provided all the time during the cooler months and at the time these observations commenced, it was turned off only during periods of exceptional heat during the summers. It is clear that a certain minimum of both heat and light is necessary for the formation of inclusions, and it is possible that the form of these might be modified by changing the balance between the amounts of heat and light made available to the plants. It is not suggested that variation could not be brought about in other ways such as by the supply of nutrients available. This is however unlikely to be the cause of the variations recorded here as all the experimental plants described were grown in rather rich soil.

If the type of inclusion body is controlled by environmental conditions, it would be expected that those new types now described would have been found previously by workers

in countries which have a greater amount of sunshine than is experienced here. Except the 'raphides' no fibrous forms have ever been mentioned. The amorphous bodies noted by Hirayama & Yuasa (1937) may be similar to those described in this paper. It is possible that other types have occurred but have been destroyed or distorted by the technique employed in examining infected cells. Most of the cytological observations on tobacco mosaic disease have been made on fixed material and the fibrous forms would be destroyed by any but the least acid fixatives. The new amorphous forms (Pl. 14, figs. 2–6) would be extremely difficult to fix in the large vacuolated cells which usually contain them and would in all probability be misinterpreted.

It has never been possible to find any differences between the hexagonal crystals usually produced by the three strains. These crystals and the more usual type of amorphous inclusion body induced by aucuba mosaic virus obviously contain some constituent in common. A variety of previously unrecorded forms has now occurred. These types are similar with all three virus strains. Moreover, they are often derived from pre-existing bodies of the better known types. It appears that, although they differ in morphology, there can be little essential difference between any of the inclusions formed by these three virus strains.

Summary

According to previous accounts tobacco mosaic virus regularly induced striate material and amoeba-like inclusions and occasionally raphides in the host cells; enation mosaic virus gave striate material and amoeba-like *X*-bodies; whilst aucuba mosaic virus induced either striate material or a large amorphous inclusion which later gave rise to striate material. A spike-like body recorded in the early descriptions of aucuba mosaic disease had not been seen for some years. In 1940, a variety of new forms were induced by all three strains. These new forms were mostly fibrous. The spike-like body reappeared, spindle-shaped bodies, masses of short needle-like fibres and extremely long coiled fibrous forms occurred. New amorphous forms were also found. All these arose either directly or from pre-existing inclusions of the previously recorded types. Variation in the inclusions produced is not due to mutation of the virus. The type of inclusion is to some slight extent determined by the host plant but seems to be largely controlled by the amount of light and heat available to the host.

REFERENCES

Bawden, F. C. & Pirie, N. W. (1937). The isolation and some properties of liquid crystalline substances from solanaceous plants infected with three strains of tobacco mosaic virus. *Proc. Roy. Soc.* B, **123**, 274.

Bawden, F. C. & Sheffield, F. M. L. (1939). The intracellular inclusions of some plant virus diseases. *Ann. appl. Biol.* **26**, 102.

Beale, H. Purdy (1937). Relation of Stanley's crystalline tobacco virus protein to intracellular crystalline deposits. *Contr. Boyce Thompson Inst.* **8**, 413.

Best, R. (1937). Visible mesomorphic fibres of tobacco mosaic virus in juice from diseased plants. *Nature, Lond.*, **139**, 628.

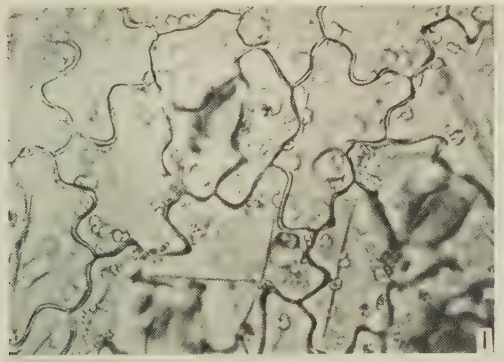
Goldstein, B. (1926). A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. *Bull. Torr. Bot. Cl.* **53**, 499.

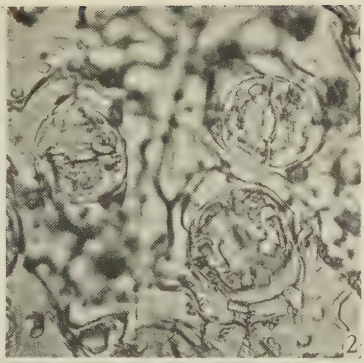
Hirayama, S. & Yuasa, A. (1937). Occurrence of inclusion bodies in the guard cells of the stomata of mosaic-tobacco plants. *Phytopath. Soc. Japan*, **6**, 305.

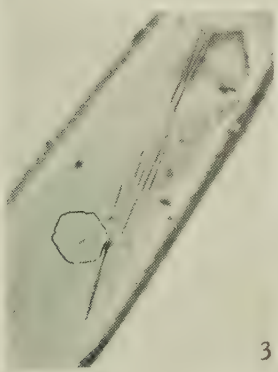
Iwanowski, D. (1903). Über die Mosaikkrankheit der Tabakspflanze. *Z. PflKrank.* **13**, 1.

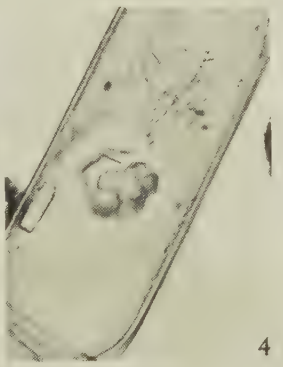
Kassanis, B. (1939). Intranuclear inclusions in virus infected plants. *Ann. appl. Biol.* **26**, 705.

Sheffield, F. M. L. (1931). The formation of intracellular inclusions in solanaceous hosts infected with aucuba mosaic of tomato. *Ann. appl. Biol.* **18**, 147.

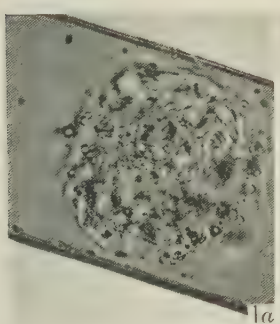


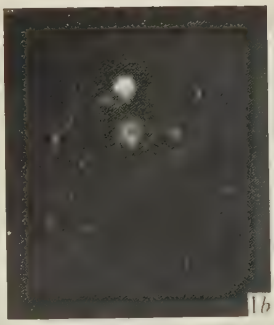


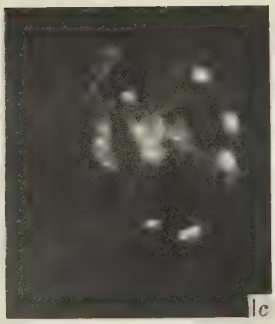


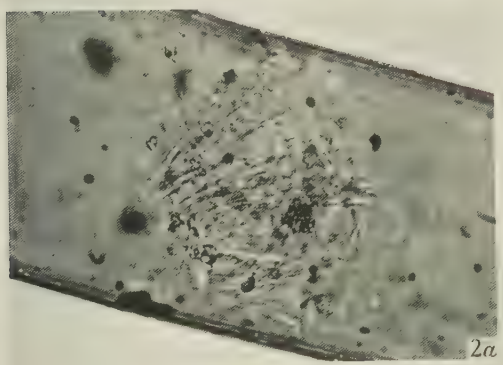


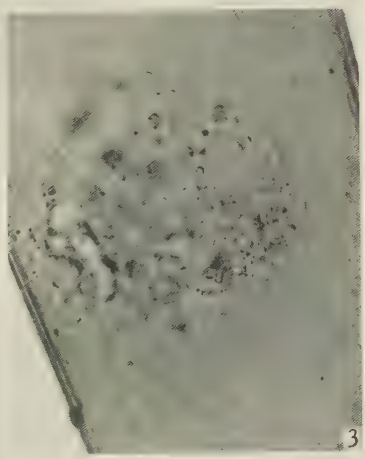
KASSANIS AND SHEFFIELD—VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS (pp. 360–7)

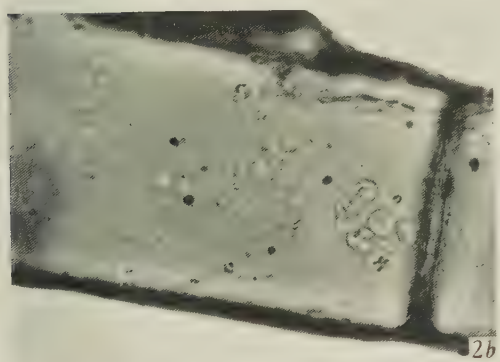


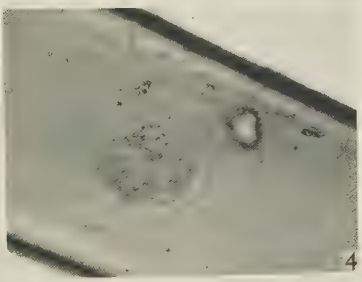


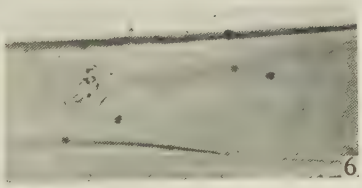












KASSANIS AND SHEFFIELD—VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS (pp. 360–7)

Sheffield, F. M. L. (1936*a*). The role of plasmodesms in the translocation of virus. *Ann. appl. Biol.* **23**, 506.
—— (1936*b*). Histology of the necrotic lesions induced by virus diseases. *Ann. appl. Biol.* **23**, 725.
—— (1939*a*). Micrurgical studies on virus infected plants. *Proc. Roy. Soc.* B, **126**, 529.
—— (1939*b*). Some effects of plant virus diseases on the cells of their hosts. *J. Roy. micr. Soc.* **59**, 149.
—— (1941). The cytoplasmic and nuclear inclusions associated with severe etch virus. *J. Roy. micr. Soc.* **61**, 30.
Smith, J. Henderson (1930). Intracellular inclusions in mosaic of *Solanum nodiflorum*. *Ann. appl. Biol.* **17**, 213.
Soukoff, K. S. & Vovk, A. M. (1938). Mosaic of cultivated cereals and how it is communicated in nature. *C.R. Acad. Sci. U.R.S.S.* **20**, 745.
Stanley, W. M. (1936). Studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. *Phytopathology*, **26**, 305.

EXPLANATION OF PLATES 13 AND 14

All preparations were made from unstained living material. Pl. 13, figs. 1 and 2, are epidermal strippings: all other figures show parts of hair cells from petiole or lamina. All photographs were taken with a Leitz Makam camera. A Leitz 6L objective (N.A. 0·65) was used in conjunction with a Leitz 10× periplanatic ocular giving a magnification of 450× on the negative. Contact prints are reproduced without alteration in size. The source of illumination is given in brackets after the description of each figure: 'MV' signifies a mercury vapour lamp, and 'Monla', a Leitz lamp of that name; the numbers refer to Wratten filters.

Plate 13

Fig. 1. Tobacco infected with tobacco mosaic virus. Long spike-like bodies, plastids and a few crystalline inclusions are seen. (MV, 62.)

Fig. 2. Tobacco infected with tobacco mosaic virus. Spike-like bodies and small crystalline inclusions are present in the guard cells of the stomata. (MV, 62.)

Fig. 3. Tobacco infected with tobacco mosaic virus. A crystalline inclusion and a mass of long fibres occupy the centre of the cell. (MV, 62.)

Fig. 4. Tobacco infected with tobacco mosaic virus. A mass of needle-like fibres is present; lying against the cell wall is a hexagonal crystal which in edge view appears rectangular; in the centre is a shapeless mass formed by the aggregation of hexagonal crystals. (MV, 62.)

Fig. 5. Tomato, 8 months after infection with enation mosaic virus. Hexagonal crystals in edge view show distinct striations although untreated with acid. (MV, 62.)

Fig. 6. Tomato infected with aucuba mosaic virus. Long fibres curve to form a figure 8. (Monla, 58 and 22.)

Fig. 7. Tomato infected with aucuba mosaic virus. A spindle-shaped body appears to be an aggregation of long spike-like fibres. (Monla, 58 and 22.)

Fig. 8. Tomato infected with aucuba mosaic virus. A long doubly refractive fibre lies parallel to the cell wall and close to it. (Monla, between crossed Nicol prisms.)

Plate 14

Fig. 1*a*. Tomato infected with aucuba mosaic virus. An inclusion body (approx. 70 μ diam.) contains amorphous material and a few small crystals. (Monla, 58 and 22.)

Fig. 1*b*. Inclusion in fig. 1*a*, seen between crossed Nicol prisms; the crystals and a little of the granular material are birefringent. (Monla, between crossed Nicol prisms.)

Fig. 1*c*. As fig. 1*b*, 30 hr. later. More birefringent material is apparent.

Fig. 2*a*. Tomato infected with aucuba mosaic virus. A large amorphous body gives rise to fine needle-like fibres and also to hyaline spherical bodies, each containing a few highly refractive granules. (Monla, 58 and 22.)

Fig. 2*b*. Another part of the cell shown in fig. 2*a*. Large numbers of small hyaline spheres each containing a few granules float in the cell sap. (Monla, 58 and 22.)

Fig. 3. Tomato, 8 months after infection with enation mosaic virus. A large mass seems to consist of amorphous material and small hyaline spheres, some of which contain minute round or rectangular particles: from the mass project hyaline bodies. (MV, 62.)

Fig. 4. Tomato, 7 months after infection with tobacco mosaic virus. A body is partly hyaline and partly contains granular material. (MV, 62.)

Fig. 5. Tomato, 8 months after infection with enation mosaic virus. An inclusion is very similar to that shown in fig. 4: hyaline bodies also are present. (MV, 62.)

Fig. 6. Tomato, infected with aucuba mosaic virus. The cell contains an amoeboid body similar in appearance to the *X*-bodies of tobacco and enation mosaics: part of a long fibre also seen. (Monla, 58 and 22.)

(*Received* 18 *March* 1941)

PHYSIOLOGICAL RELATIONSHIPS BETWEEN INSECTS AND THEIR HOST PLANTS

II. A PRELIMINARY STUDY OF THE EFFECTS OF APHIDES ON THE CHEMICAL COMPOSITION OF CABBAGE AND FIELD BEANS

By A. C. EVANS

Rothamsted Experimental Station, Harpenden

(With 2 Text-figures)

The difficulties of arriving at satisfactory estimates of losses due to the depredations of insect pests of crops have often been pointed out (e.g. Gimingham, 1939). In addition to the direct reduction of yields, insects may also be the cause of changes in chemical composition, a factor likely to be of particular importance in crops, such as sugar beet, grown as a source of special substances. Very little work has been done on this aspect of the problem. Leonard & Turner (1918) showed that the larvae of the beetle *Cerotoma trifurcata* Forst. reduce the amount of nitrogen fixed by the cow-pea by reducing the weight and nitrogen content of the root nodules. Hartzell (1913) studied the sugar and acid contents of Concord grapes obtained from vineyards infested by the grape leaf-hopper *Typhlocyba comes* Say. Grapes obtained from those sections of the vineyards sprayed with nicotine had higher sugar contents and lower acid contents than those obtained from unsprayed infested sections. Johnson (1934) carried out a detailed investigation of the effects of the potato leaf-hopper, *Empoasca fabae* Harris, on the chemical composition of alfalfa leaves. His results are compared below with those obtained in the present investigation.

Brevicoryne brassicae L. on cabbage

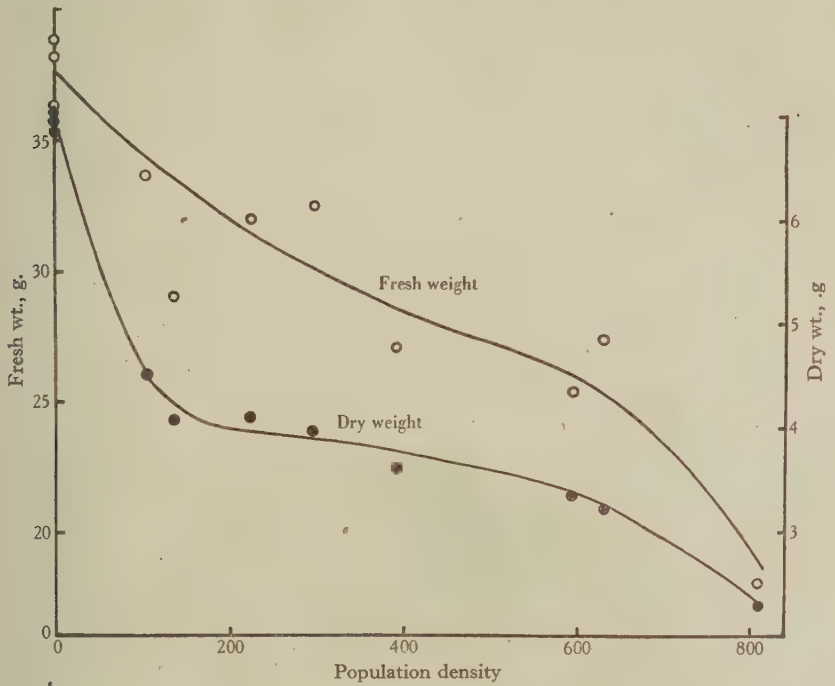
The effect of the cabbage aphis *Brevicoryne brassicae* L. on the chemical composition of cabbage (var. Sutton's 'Tender and True') has been chiefly studied. Material for analysis was obtained in the following way. Sixteen small cabbages were infested on 1 June 1937 with varying numbers (1–12) of aphides and four were kept uninfested as controls. Reproduction occurred chiefly on the small centre leaves. These were therefore cut out and left to wither in situ and the aphides then wandered on to the remaining leaves but did not produce an even infestation over the whole plant. The proportion of smaller leaves infested was not noticeably higher than that of the larger leaves. As some leaves on the plants were heavily and others only lightly infested, the plant was not prepared as a unit for analysis. Instead, the leaves were classified as highly, moderately, lightly and uninfested (control plants) leaves and three random samples of five leaves were collected of each type. The aphides were rapidly brushed off the leaves into alcohol for future enumeration and the leaves were weighed and dried to constant weight in an oven at 98° C. This technique is admittedly not as satisfactory as one which would involve analysis of the whole plant, but in a preliminary investigation its use is justified.

The degree of infestation is expressed by dividing the number of aphides (all instars) in

the sample by the fresh weight of the sample, the dividend so obtained being termed the population density, i.e. the number of aphides per gram of leaf.

Chemical analyses were carried out by methods already described (Evans, 1939).

Fig. 1 shows that there is a marked difference in the effect of increasing population density on the fresh and on the dry weights of the samples of cabbage. A reduction of



Text-fig. 1. The effect of population density of *B. brassicae* on the fresh and dry weight yields of samples of five leaves.

TABLE 1. *The effect of population density of* Brevicoryne brassicae *on the chemical composition of cabbage (expressed on a dry weight basis)*

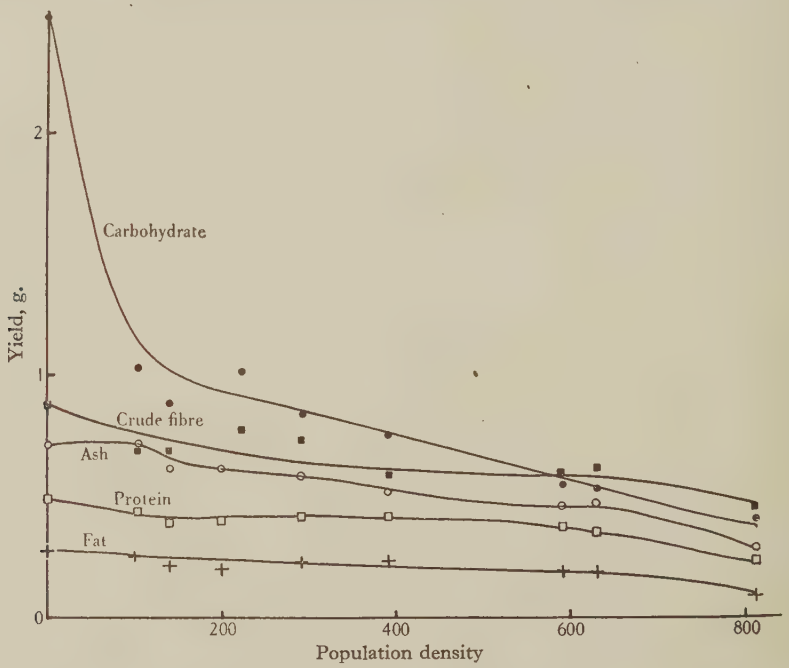
Population density	0	105	136	224	298	391	596	634	813
% dry weight	18·4	13·5	14·1	12·8	12·1	13·1	13·2	11·7	12·7
% ash	10·3	16·0	14·9	14·9	15·0	14·2	13·4	14·6	12·8
% fat	3·86	5·50	5·32	4·95	5·96	6·59	5·68	5·90	4·56
% protein N	1·12	1·52	1·49	1·58	1·67	1·81	1·78	1·68	1·56
% non-protein N	0·65	0·54	0·58	0·56	0·67	0·63	0·60	0·61	0·62
% reducing sugar	13·1	7·7	7·8	10·7	4·2	6·4	2·9	6·4	5·5
% sucrose	2·4	2·8	0·8	2·4	6·0	4·1	4·7	2·4	3·6
% starch	9·5	4·2	4·2	4·5	3·3	3·2	3·2	2·0	2·3
% acid-hydrolysable substances	11·0	8·3	8·3	7·2	7·7	7·1	5·4	5·9	5·8
% crude fibre	12·5	15·3	16·6	18·1	18·5	16·2	17·4	19·2	19·2

20% in the fresh weight is brought about by a population density of about 250 while a similar percentage reduction in dry weight is brought about by a population density of 50. This great reduction in the yield of dry material compared with that of fresh material is due to a sudden decrease in the percentage of dry matter in the infested plants (see Table 1).

Table 1 also shows the change in composition of the dry matter with increasing population density.

In general, the percentages of ash, fat and protein nitrogen increase to a maximum at a population density of 300–400 and then decline to a figure still higher than that of uninfested material. The percentage of crude fibre increases to a maximum at a population density of 600–800. The percentages of the several carbohydrate fractions decrease with increasing population density except perhaps that of sucrose, the figures for which are very erratic.

Fig. 2 shows the effect of these changes in chemical composition on the yields of carbohydrate (reducing sugars, sucrose, starch and acid hydrolysable substances), protein



Text-fig. 2. The effect of population density of *B. brassicae* on the yields of carbohydrate, protein, fat, ash and crude fibre.

(N × 6·25), fat, ash and crude fibre, obtained from each sample. The most noticeable effect is on the yield of carbohydrate which falls rapidly at first and then more slowly. The yields of crude fibre and ash fall fairly steadily with increasing population density while those of protein and fat are hardly affected by an increase in population density up to 400.

The effect of *Empoasca fabae* on the composition of alfalfa as found by Johnson (1934) is the opposite of that recorded above for *B. brassicae*, *E. fabae* causing an increase in the percentages of dry matter, reducing sugar, starch and acid hydrolysable substances and a reduction in the percentage of protein N (see Table 2). Johnson clearly showed that the feeding habits of *E. fabae* account for the increased carbohydrate and reduced protein nitrogen contents of attacked alfalfa. The leaf-hoppers, when feeding, puncture and block the conducting tissues in the petiole thus greatly impeding the removal of carbohydrates

from the leaves and the supply of nitrogenous salts to the leaves. With *B. brassicae* the cause of the great reduction in carbohydrate is not known. That it is perhaps due to a specific effect of a substance injected by the aphis on the mechanism of carbohydrate production is suggested by the fact that the production of fat and protein is affected but little by a low infestation which reduces carbohydrate production seriously.

TABLE 2. *Effect of* Empoasca fabae *on the composition of alfalfa.* (*Results calculated to the same bases as Table* 1 *from data given by Johnson* (1934))

	% dry weight	% reducing sugar	% sucrose	% starch	% acid-hydrolysable substances	% protein N
Not attacked	34·5	0·8	1·9	1·6	8·1	3·72
Attacked	37·0	3·4	2·7	7·2	16·6	2·55

APHIS FABAE SCOP. ON FIELD BEANS

Some data have also been obtained on the effect of *Aphis fabae* Scop. on the composition of field beans (Table 3). Material for analysis was collected on 2 Aug. from naturally infested crops of winter and spring beans. The plants were graded as clean, dirty and very dirty according to the appearance of the foliage since the aphides by this date had almost disappeared. In this case, aphis infestation has not materially affected the chemical composition of the beans. Similar figures were obtained for winter beans.

Thus it is seen from the instances recorded in this paper that insects may or may not affect materially the chemical composition of the plants on which they feed. A thorough knowledge of these effects might prove of value in assessing accurately the damage caused by pests.

TABLE 3. *The effect of* Aphis fabae *on the chemical composition of spring beans.* (*Expressed on a dry-weight basis*)

	Clean		Dirty		Very dirty	
% dry weight	23·6	24·9	22·7	26·9	19·2	23·6
% ash	4·3	4·4	4·6	3·9	5·3	4·6
% fat	3·2	2·9	4·2	3·7	3·8	3·9
% crude protein	23·3	22·8	24·5	24·1	28·9	25·4
% carbohydrates sol.	58·5	63·0	57·6	61·6	46·3	59·9

SUMMARY

Infestation of cabbage by the aphis, *Brevicoryne brassicae*, caused a marked decrease in the amount of carbohydrate synthesized but smaller decreases in fat, crude protein and other constituents. Infestation of field beans by the aphis, *Aphis fabae*, did not have any great effect on the chemical composition of the crop.

REFERENCES

EVANS, A. C. (1939). The utilisation of food by the larva of the Buff-tip, *Phalera bucephala* (L.). *Proc. R. ent. Soc. Lond.* A, **14**, 25.

GIMINGHAM, C. T. (1939). Crop pests and diseases. *Sci. Progr.* **33**, 655.

HARTZELL, F. Z. (1913). The grape leaf-hopper. *Bull. N.Y. St. agric. Exp. Sta.* no. 359.

JOHNSON, H. W. (1934). Nature of injury to forage legumes by the potato leaf-hopper. *J. agric. Res.* **49**, 379.

LEONARD, L. T. & TURNER, C. F. (1918). Influence of *Cerotoma trifurcata* on the nitrogen-gathering functions of the cow-pea. *Amer. Soc. Agron.* **10**, 256.

(*Received* 1 *May* 1941)

HOST SPECIALIZATION OF *ANGUILLULINA PRATENSIS* (DE MAN)

I. ATTRACTIVENESS OF ROOTS

By C. H. GADD and C. A. LOOS

Tea Research Institute, St Coombs, Talawakelle, Ceylon

A knowledge of the susceptibility and resistance of certain plants, or plant varieties, to nematode pests is of considerable importance to plant pathologists. Undoubtedly, the ultimate test of resistance or susceptibility of any species or variety of plant is a field test on infected areas. Where annual plants are concerned it is often practicable to proceed directly to field trials, but when trees or shrubs have to be tested the time and space occupied by such trials become of greater importance, and there is need for preliminary experiments which will provide a *measure* of the resistance or susceptibility of the planting material.

Steiner (1925) expressed the opinion that all the endeavour towards the breeding of immune or resistant crops had not led to any notable result because of the complete ignoring of the host selection problem which is of fundamental importance. He distinguished three types of problem connected with host selection and host specialization of plant parasitic nematodes: (1) *Host attraction*, non-attraction and repellency of nematodes; (2) *Host resistance*, i.e. opposition to the entrance of nematodes by some mechanical or perhaps chemical means; and (3) *Host immunity* as exhibited by the nematodes being attracted to the host, entering and living in it, yet doing no perceptible harm. The terms 'resistance' and 'immunity' here have a somewhat restricted meaning, but it will be apparent that a search for so-called immune or resistant species or varieties of plants must involve a study of these three problems.

Little is known concerning host resistance and host immunity, but the problem of host attraction has received considerable attention. It is now generally accepted that by means of certain sense organs, the amphids, nematodes recognize their hosts owing to the exudation by the roots of chemical substances which are carried in the soil water. By moving from regions of low concentration to those of higher concentrations the nematodes are led directly to the exuding roots. Little is known of the nature of the active root exudates or whether they are specific for each plant species or variety. Linford (1939) described methods for demonstrating by direct microscopic observations the attractiveness to certain nematodes of growing roots and of excised stem and leaf tissues, both fresh and decomposing. His experiments probably indicate chemotropic responses to more than one chemical stimulant.

In the course of other work it became necessary to collect information regarding the host specialization of *Anguillulina pratensis* (De Man) which is an important parasite of tea bushes. It was found preferable to divide the problem into two parts, not three as suggested by Steiner, and to devise methods of measuring the interactions between host and parasite. We have therefore studied the reactions of *A. pratensis* towards the roots of various plants in an attempt to evaluate (1) the attractiveness of the roots to the nematodes, and (2) the behaviour of the nematodes after entry into the roots.

The attractiveness of a root to an internal parasite such as *A. pratensis* is best measured by counting the eelworms entering the root under definite conditions in a given time. Such a determination includes a measure not only of attraction but also of resistance as defined by Steiner. However, there are few, if any, cases on record of endoparasitic eelworms being attracted to particular roots and then being unable to enter owing to host resistance. In the event of eelworms failing to enter roots, the failure may be worthy of further study. It may be due to non-attraction or even repellency of the roots; or to physical inability of the eelworms to penetrate the roots after reaching them; or to other causes. Instances of non-entry into attractive tissues are discussed later. But in general, if the above interpretations of negative results are borne in mind, the entry of internal parasites into roots offers a fair measure of the attractiveness of those roots to the parasite.

Methods

Anguillulina pratensis was isolated from lesions in large tea roots by methods described previously (Gadd & Loos, 1941) and it may fairly be assumed that the worms had fed on tea roots for many generations. For experiments with adult worms, females only were used. Freshly hatched larvae were obtained by hatching the eggs in damp sand. Larvae could also be obtained by allowing a suspension of eelworms and eggs obtained by washing the separated wood and cortex of infested roots to stand for 2 days. During this time there is a marked increase in the number of small larvae which may be picked up with a fibre needle. *Heterodera marioni* larvae were obtained by hatching, in water, egg masses collected from *Tephrosia Vogelii* roots. It is very probable that this species had also lived in *Tephrosia* roots for many generations.

In the experiments described here, seedling roots only were used. The seeds were germinated on damp blotting paper or wet sand, and were used for experiment when the primary roots were 1–2 in. long. Unless otherwise stated, the roots were not cut off till they were about to be macerated, preparatory to examination. When root examination was delayed for a period after infestation, the seedlings were planted in pots of sterilized sand and kept in the laboratory, the temperature of which varied from 64 to 83° F.

The following methods of infestation were used:

(1) *Wet sand.* A drop of water was placed on a microscope slide or other suitable piece of glass and to it was added a given but arbitrary number of worms: 3 worms for experiments with *Anguillulina pratensis* and 5 with *Heterodera marioni*. At this stage, the contents of the water may easily be checked by low magnification. After adding sufficient fine dry sand to absorb the water, a recently germinated seedling was placed on the slide so that its root tip rested on the damp sand. With more dry sand a heap was made to cover the apical part of the root which was then wetted sufficiently to support root growth. The seedlings were then placed in Petri dishes with wet blotting paper adhering to the lids for one or two days before examination. The sand used was washed river sand, sieved through a 60 mesh sieve.

(2) *Water.* The worms were placed in water as before and the seedling placed on the slide so that its root tip rested in the water. The slides were then enclosed in damp chambers for 24 hr. For some experiments 1 worm only was placed in the water; then, the infested plants were not, as a rule, examined for a week or more, but were maintained in pots till ready.

Examination. After removal from sand the roots were washed, severed just below the seed, macerated in a mixture of equal parts of 10% chromic and 10% nitric acids, washed again, mounted in caustic potash and lightly squashed under the coverglass as described elsewhere (Gadd & Loos, 1941). The number of worms within the roots can then easily be ascertained by low magnification.

The number of worms found within the roots, expressed as a percentage of the number used, gives a measure of the attractiveness of the roots. When a standard number of worms is used per root, a better measure is the mean number of worms found per root, as the standard deviation and other values can be calculated from the frequency distribution for comparison of results.

Discussion

Variability of results. Where 3 or 5 worms were used per root, 10 roots as a rule were taken for each experiment. When an experiment was repeated, in some cases a month or more later, eelworms from a different tea root, often from a different tea bush, were used. Nevertheless, the results obtained were consistent. For instance *Tephrosia Vogelii* was tested in sand for 24 hr. on four different occasions; 10 roots were used on each occasion and 3 worms were included in each heap of sand. The mean entries per root together with their standard errors were respectively: $2{\cdot}2 \pm 0{\cdot}249$, $2{\cdot}2 \pm 0{\cdot}291$, $1{\cdot}9 \pm 0{\cdot}314$ and $1{\cdot}9 \pm 0{\cdot}348$. The differences observed between the means are not of statistical significance, and the results of these experiments have therefore been united in the appropriate row in Table 1 where the mean is shown as $2{\cdot}05 \pm 0{\cdot}147$. Other results have been similarly united in the tables.

Table 1. *Attraction of* Anguillulina pratensis *females to seedling roots in damp sand. Three adults were used for each root and examinations were made on removal from the sand except where otherwise stated*

Seedling	Time in sand hr.	No. of worms used	No. of worms entered	% infestation	No. of roots used	No. of roots entered	Mean content	S.E. mean
Tephrosia Vogelii	24	120	82	68	40	37	2·05	0·147
	48	60	41	68	20	19	2·05	0·198
(excised)	24	120	53	44	40	34	1·32	0·136
	48	90	68	76	30	30	2·27	0·143
(dead)	24	60	5	8	20	4	0·25	0·123
	48	90	1	1	30	1	0·03	0·033
(rotting)	24	60	0	0	20	0	0	—
	48	60	0	0	20	0	0	—
Tea	24*	72	20	28	24	14	0·83	0·167
	48†	102	61	60	34	32	1·79	0·145
	48‡	72	40	56	24	22	1·67	0·155
Turnip (Earl's White)	24	90	16	18	30	11	0·53	0·157
	48	60	25	42	20	16	1·25	0·190
Crotalaria anagyroides	24	30	22	73	10	10	2·2	0·20
	48	90	56	62	30	28	1·87	0·157
Desmodium gyroides	24	60	17	28	20	10	0·85	0·235
	48	60	39	65	20	19	1·95	0·198

* United results from examinations after 1, 7 and 21 days.
† Examined after 7 days. ‡ Examined after 21 days.

Mass attraction. Godfrey & Oliveira (1932), working with *Heterodera marioni* larvae, came to the conclusion that mass action is contributory to penetration of roots because a large number of larvae frequently penetrate a root on one side and at or near the same point. Mass action may here imply a preference by the larvae for taking the easiest route, namely the passages already made by the forerunners, or it may indicate a greater exudation of the attractive substance resulting from wounds made by the first comers. It does not necessarily suggest an inability of the individual larvae forming the mass to enter by their own unaided efforts. Tyler (1933), however, found a great difference in the ability of *H. marioni* larvae to penetrate plant tissue, and there is a possibility that some individuals may not be able to penetrate roots except with the aid of others. For this reason, it is advisable to keep the number of individuals in each heap of sand relatively small, in order

to avoid loading the results unduly and making them more a measure of mass action than of attraction. In the experiments here described, using 3 adults of *Anguillulina pratensis* or 5 larvae of *Heterodera marioni* for each experiment, no indication of mass attack was observed during examination of the numerous roots tested.

Infestation media. Tables 1 and 2 show results obtained when infestations were obtained by the sand and water methods respectively, using 3 female *A. pratensis* per root. Greater percentage infestations and higher mean contents of roots were observed after 24 hr. when water was the medium than when sand was used; possibly the attractive substance diffuses more rapidly through a small quantity of water than through a mound of damp sand. Other results are given in Table 3 of infestations made in water, but in these experiments 1 worm only was used per root and examinations were not made till the seventh day

TABLE 2. *Infestation of seedling roots by female* Anguillulina pratensis *in water for 24 hr. Three adults were used for each root and examinations were made on removal from damp chamber*

Seedling	No. of worms used	No. of worms entered	% infestation	No. of roots used	No. of roots entered	Mean content of roots	S.E. mean
Tephrosia Vogelii	90	73	81	30	29	2·4	0·149
(excised)	30	23	77	10	9	2·3	0·334
(dead)	60	7	12	20	6	0·35	0·279
Desmodium gyroides	30	26	87	10	10	2·6	0·218
Turnip	120	81	68	40	39	2·03	0·127

TABLE 3. *Infestation of seedling roots by female* Anguillulina pratensis *in water for 24 hr. One adult was used for each root*

Seedling	No. of worms used	Entries		Worms leaving after egg laying		Examined
		No.	%	No.	%	
Tephrosia Vogelii	93	64	69	0	—	7 days
	93	73	78	2	3	21 ,,
Desmodium gyroides	46	42	91	1	2	7 ,,
	42	36	86	22	61	21 ,,
Turnip	69	40	58	1	2·5	7 ,,
	69	28	41	4	14	21 ,,
Crotalaria anagyroides	35	28	80	0	—	7 ,,
	61	31	51	1	3	21 ,,
Tea	43	9	21	0	—	1–4 weeks

or later. Again, the percentage entries into the roots were high except for tea (21 %), which is perhaps somewhat surprising, as tea was the host plant from which the worms were originally obtained. *Desmodium gyroides*, which from experience of its growth on infested soils, is regarded as highly resistant or immune to attacks of *Anguillulina pratensis* here gives the highest percentage of infestation (91 %). It was noticed, however, that although the experiments were carried out in damp chambers to stop evaporation, the water on the slides disappeared, probably by absorption by the roots, except when tea seedlings were the experimental plants.

When eelworms are placed in a drop of water on a slide, they can be seen to move freely, yet are unable to leave the water owing to its surface tension. As the water evaporates, the

worms are drawn closer together until they form a tightly packed mass, enclosed in a thin film of water. A few minutes after the last trace of water has disappeared the eelworms are dead, when the species used for the experiment is *A. pratensis* and the relative humidity of the room is about 70%. When a root tip is in the water in addition to the eelworms, the worms are drawn mechanically to the root as the water disappears. It seems probable that the low percentage of infestation of tea seedlings obtained by the water method is largely due to the fact that the water is not rapidly absorbed by the roots and consequently the worms are not drawn mechanically to them. The water method, therefore, does not afford a reliable measure of attraction, though it may be used to demonstrate the ability of worms to enter specific roots.

Wet sand allows more freedom of movement to the worms, which have no difficulty in leaving it, particularly when a fall in temperature causes the deposition of a film of moisture on the slide. If a heap of wet sand containing 10 or 12 worms is placed in a damp chamber overnight and examined in the morning, some of the worms, often 5 or 6, will be found on the slide some distance from the sand. They can usually be located by their tracks visible on the misted surface of the slide. Linford (1939) found that nematodes grouped more

TABLE 4. *Infestation of seedling roots by* Anguillulina pratensis *larvae. Three larvae were used for each root and examinations were made after* 48 *hr.*

Seedling	Medium	Examined	No. of worms used	No. of worms entered	% infestation	No. of roots used	No. of roots entered	Mean content of roots	S.E. mean
T. Vogelii A	Sand	48 hr.	78	36	46	26	19	1·38	0·215
,, B	Sand	48 ,,	90	37	41	30	20	1·23	0·196
,, A	Water	24 ,,	90	67	75	30	30	2·23	0·115
Tea A	Sand	48 ,,	105	10	10	35	9	0·29	0·088

A, larvae hatched in sand. B, larvae collected from a suspension.

rapidly around roots in sand than in soil. He attributed this to the freer movement of nematodes allowed by the more open texture of the sand and to the probability that the attractive substances were adsorbed by soil colloids thereby reducing the intensity and extent of attraction. Sand, therefore, appears to be the more suitable medium for attraction tests.

Tests with Anguillulina pratensis *larvae.* The results of a few experiments with newly hatched larvae of *A. pratensis* are given in Table 4. In the experiments marked A, the eggs were collected from tea cortex and hatched in damp sand, so that the larvae had no opportunity of feeding on any tissue before being tested with seedling roots. In that marked B, small larvae were collected from a suspension obtained from tea cortex which had been allowed to stand for 2 days. These larvae also were recently hatched but there was no certainty that they had not fed on tissue before test. It is apparent however from the tests on *Tephrosia Vogelii* in sand for 48 hr. that the reactions of both lots of larvae to the roots were equal.

Again, a much higher percentage of infestation was obtained when water was the medium. The mean content of the roots infested in water was not significantly different from that obtained when adults were used; the mean difference is $0{\cdot}2 \pm 0{\cdot}16$. When, however, sand was the medium the entries made by larvae after 48 hr. were significantly less than those made by adults. The observed differences were $0{\cdot}67 \pm 0{\cdot}292$ for *Tephrosia* and $1{\cdot}5 \pm 0{\cdot}169$ for tea.

The occurrence of such differences is not unexpected since what has been measured as attraction includes not only the attractiveness of the root but also the ability of the worms to enter. Entry into a root must, to some extent, be a matter of physical strength. It is therefore possible that the fewer entries made by larvae in sand result from their relative weakness rather than from their inability to find the roots. No energy is expended by larvae to reach the roots in water.

Effect of time on attraction. There was no increase in the number of entries into *Tephrosia* and *Crotalaria* roots in sand when the time was extended from 24 to 48 hr., but definite increases occurred when turnip, tea and *Desmodium* were left for the longer period (Table 1). The attractiveness of roots to eelworms in tests such as these will depend not only upon the nature of the exuded substance, which may determine whether a particular species will be attracted or not, but also upon the rate at which it is exuded and transported or diffused. If the rate of exudation is relatively slow, the eelworms would be only slightly attracted at first, but as more of the attractive substance is exuded with the passage of time its attractiveness would become more powerful and would extend through a greater volume. If then the same attractive chemical substance is exuded by *Tephrosia*, turnip and tea the observed results may be accounted for if the *Tephrosia* roots exude it more rapidly than turnip or tea.

Growth and attraction. When *Tephrosia* roots were severed from the seed before being tested in sand, they became less attractive during the first 24 hr. (Table 1). During the second day the mean root content increased from 1·32 to 2·27, a value very close to that observed for unsevered roots, viz. 2·05. When excised *Tephrosia* roots were tested by the water method (Table 2) the mean entry into the roots (2·3) was as large as that observed (2·0) when the roots were not severed.

During these tests the excised roots increased in length but not to the same extent as the unsevered roots. Excision of the root does not stop growth but diminishes it. Associated with the reduced rate of growth is a reduction in the attractiveness of the roots as shown by the experiments in sand. An explanation has already been offered why the experiments in water should not give similar results, viz. that by that method the worms are drawn to the roots mechanically and not chemotropically. The extension of growing roots puts a limit to the period during which experiments may be continued by the sand method, as at the end of 48 hr. the roots, as a rule, have pushed themselves out of the sand.

Further evidence of a correlation existing between the rate of growth of roots and their attractiveness to *Anguillulina pratensis* is supplied by experiments with dead roots.

Attraction to dead roots. Seedlings of *Tephrosia Vogelii* were immersed in boiling water for a few minutes; the roots were then severed and used for attraction tests. Their attractiveness to *Anguillulina pratensis* was almost completely lost, the mean entry per root falling from 2·05 for living roots to 0·25 for dead ones (Table 1). Similar results were observed when water was used as the medium for infestation (Table 2). The mean content of these roots was 0·35 as compared with 2·4 for living roots. There is no apparent reason why a dead root, freshly killed, should offer greater mechanical resistance to entry than a living root. A number of chemical changes occur within a root on its death and probably the attractive substance is removed or destroyed. Consequently, the roots become unattractive to eelworms at least for the time being, even when water is used as the medium.

Attraction to decaying roots. Linford (1939) found that tissues rendered unattractive to

eelworms by cooking became attractive as they rotted. In order to ascertain whether similar attraction could be demonstrated by these methods, *Tephrosia* roots killed in boiling water as before were placed in damp sand for 48 hr. to rot. At the end of that time they had unpleasant odour indicative of decay. They were then placed in mounds of damp sand, e with 3 female *Anguillulina pratensis* for test. The results (Table 1) show that rotting r are no more attractive than freshly killed roots. These results do not necessarily contra Linford's observations, as his criterion was not entry into the decaying tissues but c gregation around them.

Attraction of Heterodera marioni *larvae to roots.* In order to compare the reaction *Heterodera marioni* larvae to *Tephrosia Vogelii* seedling roots, similar experiments w carried out with living (unsevered), excised, dead, and decaying roots. Five larvae, obtai from egg masses collected from *T. Vogelii* roots and hatched in water, were used in e heap of sand. The results are given in Table 5.

Again and for the same reason, the greatest infestation of uninjured seedlings occur when water was used as the medium. When sand was used more eelworms entered liv roots, both unsevered and excised, after 48 than after 24 hr. The increased root conte

TABLE 5. *Infestation of* Tephrosia Vogelii *seedling roots by* Heterodera marioni *larvae Five larvae were used for each root*

Condition of root	Medium	Time hr.	No. of worms used	No. of worms entered	% infestation	No. of roots used	No. of roots entered	Mean content of roots	S.E. me
Attached	Sand	24	100	21	21	20	11	1·05	0·276
,,	,,	48	200	110	55	40	37	2·75	0·211
Excised	,,	24	100	32	32	20	16	1·6	0·197
,,	,,	48	100	46	46	20	18	2·3	0·282
Dead	,,	24	100	0	0	20	0	0	—
,,	,,	48	100	0	0	20	0	0	—
Rotting	,,	48	170	1	0·6	34	1	0·03	0·025
Attached	Water	24	100	64	64	20	20	3·2	0·277

during the second day were $1·7 \pm 0·348$ for uninjured roots and $0·7 \pm 0·344$ for excised roo The former result is definitely significant but the latter is on the border line. These figu do not demonstrate the decreased attraction of excised as compared with unsevered ro as clearly as those obtained when *Anguillulina pratensis* was used, although their trend similar. When dead and rotting roots were used, *Heterodera marioni* failed to invade the during the tests. There can be no doubt from Linford's experiments that *H. marioni* larv are attracted towards decaying vegetable tissue, but from the results obtained here it evident that the attraction ceases when the worms reach the tissue as they do not enter

Attraction to living and to decaying tissues. If the view be accepted that eelworms mo from regions of low concentration always to regions of higher concentration of an attracti substance, it follows that the worms will attempt to continue their trail to the region highest concentration wherever it may be. If it is within the root and the worms have t power to penetrate the root, as *Anguillulina pratensis* and *Heterodera marioni* larvae hav they will enter the roots in order to reach the source of attraction. If on the other hand t attractive substance is formed at the surface of the root and is at its highest concentrati there, there will be no incentive for the eelworms to penetrate the tissue. It seems possib therefore that the entry of *Anguillulina pratensis* and *Heterodera marioni* larvae into livi

roots is determined by their perception of a higher concentration of the attractive substance within the root, whereas their failure to enter decaying tissue is because the highest concentration is at the surface of the root.

The attractive substance exuded by living roots is probably a by-product of growth. Such a hypothesis would account for the greater attraction to eelworms of unsevered than of excised roots, as the former grow more in a given time than the latter, and also for the fact that eelworms are attracted to and enter the growing regions of roots. As a by-product of growth the greatest concentration of the attractive substance would be expected to occur within the roots and so supply the incentive for eelworms to enter the tissues. The substance which attracts eelworms to decaying tissue is not necessarily the same as that exuded by living roots. Linford's experiments indicate chemotropic responses to more than one attractive substance. If the formation of attractive substances by decaying tissues is dependent upon an oxygen supply or if they are exuded by fungi or other organisms on the surface of the tissue, the greatest concentration would be expected at the surface.

It is suggested that the reason why certain eelworms penetrate living roots but stay outside decaying tissue although attracted to it, is to be found in the position of the highest concentration of the attractive substance and not in the chemical nature of the substance. There can be no doubt that eelworms find their food supplies readily because of their perception of and reaction to such substances. But it does not necessarily follow that all such attractive substances emanate from suitable food supplies. Evidence will be offered to show that worms enter roots which, later, they find unattractive or which prove to be unsuitable as a medium for propagation. Attraction to decaying tissues suggests the possibility that eelworms such as *Anguillulina pratensis* and *Heterodera marioni* which are often regarded as obligate parasites, may feed for a time on decaying tissues although that type of food alone may not enable the worms to complete their life cycles.

Time of examination. When infested roots are planted in sand or soil for a period before examination there is a risk that some of the worms may leave the roots. How real that risk is may be ascertained from Table 3 in which are given the results of examinations made 7 and 21 days after the beginning of the experiments, the infested plants having been grown in sterile sand during the intervals before examination. At these examinations a number of roots were found to contain eggs but no female *Anguillulina pratensis*. One root of *Desmodium gyroides* containing eggs was found at the examination after 7 days and 22 after 21 days. The presence of eggs within a root in the absence of the female that laid them is indubitable evidence that the root has been invaded though the female left before the root was examined. A female leaving a root before depositing eggs however leaves no evidence of her entry. The number of entries shown in Table 3 is therefore made up only of the number of worms present plus those roots which contained eggs but no worm.

Table 2 shows that about 87 % of the *Desmodium* roots may be expected to be infested in 24 hr. in water. The results given in Table 3 show infestations of 91 and 86 %, so there is no reason to suspect that many (if any) worms left the roots without laying eggs. But the absence of 22 worms, or 61 % of those that entered, at the examination after 21 days is clear proof that their departures occurred mainly between the seventh and twenty-first days. Here, then, is an example of roots relatively highly attractive to *Anguillulina pratensis* (Table 1) which prove distasteful after entry.

Crotalaria roots are equally, or more, attractive to *Anguillulina pratensis* than are *Desmodium*

roots (Table 1); yet at the examination made after 21 days, only 51 % of the roots were found to contain eelworms as compared with 80 % at the examination after 7 days. Possibly a number of females left roots without laying eggs or leaving other evidence of having entered them. Examination of the data concerning turnip roots also indicates the departure of worms from invaded roots. The behaviour of the worms after entering roots will be discussed more fully in Part II. It may be sufficient here to draw the conclusion that if a reliable estimate of the attractiveness of roots is to be made, the entries should be counted immediately the attraction test is completed.

Conclusions

The better method of comparing the attractiveness of roots to *Anguillulina pratensis* is by using damp sand as the medium for infestation. When 24 hr. is allowed for the period of test, the five species of plants experimented with can be grouped into two classes. *Crotalaria anagyroides* and *Tephrosia Vogelii* belong to the more attractive and *Desmodium gyroides*, tea and turnip to the less attractive class. When the count of entries is made after 48 hr., only turnip remains in the less attractive class.

In the field, the period of attraction is not limited to 48 hr. and if root attraction were the only factor affecting immunity there is no apparent reason why *Desmodium* roots should not be as severely attacked as tea roots. Field observations suggest that *Desmodium* is relatively immune whereas tea is heavily infested. Other and more important factors appear to be operative. It has already been shown that certain roots may attract the worms yet may prove so distasteful later that many leave, either before or after laying eggs. A measure of the attraction to roots alone therefore does not afford a reliable criterion of the relative susceptibility of such roots.

Summary

Methods of measuring the attractiveness of seedling roots to *Anguillulina pratensis* and *Heterodera marioni* larvae are described. As the criterion is the entry of the worms into the roots, such determinations include a measure not only of attraction to the root but also of the ability of the worms to enter. *Anguillulina pratensis* larvae do not enter roots from sand as readily as adults. The difference is attributed to their relative strengths. The attraction of roots is dependent to some extent upon their rate of growth. Neither *Anguillulina pratensis* adults nor *Heterodera marioni* enter recently killed or decaying roots though they may be attracted towards the latter. It is suggested that the entry of eelworms into living roots is due to the higher concentration of the attractive substance being within the growing tissue; in decaying tissue the highest concentration is probably at the surface. *Anguillulina pratensis* leave the roots of some species of plants after entry. A measure of the attractiveness of roots alone does not offer a reliable criterion of the true susceptibility of such roots.

REFERENCES

GADD, C. H. & LOOS, C. A. (1941). Observations on the life history of *Anguillulina pratensis*. *Ann. appl. Biol.* **28**, 39.

GODFREY, G. H. & OLIVEIRA, J. (1932). The development of the root knot nematodes in relation to root tissues of pineapple and cowpea. *Phytopathology*, **22**, 325.

LINFORD, M. B. (1939). Attractiveness of roots and excised shoot tissues of certain nematodes. *Proc. helminth. Soc. Wash.* **6**, 11.

STEINER, G. (1925). The problem of host selection and host specialization of certain plant infesting nemas and its application in the study of nemic pests. *Phytopathology*, **15**, 499.

TYLER, J. (1933). Reproduction without males in aseptic root cultures of the root knot nematode. *Hilgardia*, **7**, 373.

(*Received* 16 *April* 1941)

HOST SPECIALIZATION OF *ANGUILLULINA PRATENSIS* (DE MAN)

II. BEHAVIOUR OF THE PARASITE WITHIN ROOTS

By C. H. GADD and C. A. LOOS

Tea Research Institute, St Coombs, Talawakelle, Ceylon

The life history of *Anguillulina pratensis* (De Man) within seedling roots of *Tephrosia Vogelii* and the methods by which it was determined have been described previously (Gadd & Loos, 1941*a*). Similar methods have been used to determine the behaviour of that parasite within seedling roots of *Desmodium gyroides*, *Crotalaria anagyroides*, tea and turnip. The contents of infested roots were determined after 1 and 3 weeks for *Desmodium*, tea and turnip, and after 1, 2, 3, 4 and 5 weeks for *Crotalaria*. The results of these examinations together with relevant data from experiments with *Tephrosia*, recorded in the earlier publication, are given in Table 1.

Table 1. *The behaviour of* Anguillulina pratensis *within seedling roots*

	Tephrosia					*Crotalaria*				
Weeks	1	2	3	4	5	1	2	3	4	5
♀♀ used, no.	93	93	94	93	93	35	40	61	41	67
Observed entries	64	74	73	66	67	28	31	31	26	34
Infestation observed, %	69	80	78	71	72	80	78	51	63	51
Departures after egg laying: No.	0	1	2	1	0	0	0	1	0	4
%	0	1	3	2	0	0	0	3	0	12
Non-layers: No.	16	10	14	11	12	22	6	7	6	12
%	25	14	19	17	18	79	19	23	23	35
Layers present, no.	48	63	57	54	55	6	25	23	20	18
Mean no. of eggs per layer present	6·4	15·0	19·1	26·3	26·6	2·5	6·8	8·1	9·3	8·7
S.E. mean, eggs	0·47	0·93	1·26	1·64	1·71	0·22	0·79	1·0	0·84	1·12
Mean no. of hatched eggs per layer present	—	—	3·9	13·5	19·2	—	—	0·17	0·5	0·39
S.E. mean, larvae	—	—	0·42	0·94	1·37	—	—	0·10	0·22	0·12
Larvae as % of eggs laid 14 days earlier	—	—	61	90	100	—	—	7	7	5

	Tea		*Desmodium*		Turnip	
Weeks	1	3	1	3	1	3
♀♀ used, no.	111	87	46	42	69	69
Observed entries	62	43	42	36	40	28
Infestation observed, %	56	49	91	86	58	41
Departures after egg laying: No.	0	0	1	22	1	4
%	0	0	2	61	2	14
Non-layers: No.	11	8	8	5	22	12
%	18	19	19	14	55	43
Layers present, no.	51	35	33	9	17	12
Mean no. of eggs per layer present	4·9	26	5·0	9·0	2·8	2·8
S.E. mean, eggs	0·39	1·48	0·36	1·23	0·33	0·44
Mean no. of hatched eggs per layer present	—	2·6	—	2·5	—	0·7
S.E. mean, larvae	—	0·30	—	1·08	—	0·38
Larvae as % of eggs laid 14 days earlier	—	53	—	50	—	25

With the exception of tea seedlings, all roots were infested in a drop of water for 24 hr. using 1 adult female worm for each root. Tea roots were infested in damp sand for 48 hr. using 3 females to each root. The use of 3 worms per root reduces the number of roots giving nil results, but it has the disadvantage, when two or three worms enter the same root and lie close together, that difficulty may be experienced in determining the exact number of eggs laid by each. The difficulty does not arise when the worms are well separated within the root, as the eggs usually lie in the immediate vicinity of the worm that laid them. At each examination of the tea roots, one root was found to contain 2 worms with eggs lying in such a way that it was impossible to determine whether 1 only or both worms had contributed towards the group of eggs. Those worms have been included in the count of observed entries but are excluded from the count of layers and non-layers.

Observed entries. Some roots, particularly of *Desmodium* examined after 3 weeks, were found to contain eggs but no females. The numbers of such roots found are entered in the column headed 'Departures after egg laying', as such roots must have been invaded by females which left after laying the eggs and before the roots were examined. The number of observed entries therefore consists of the number of females present when the roots were examined together with the number of roots from which the worms had departed after egg laying. Any worms which leave the roots after entry but before laying eggs leave no direct evidence of their entry, and, consequently, cannot be counted. The percentage infestation calculated from the observed entries, therefore, does not necessarily represent the true figure. That is best estimated immediately the roots are removed from the infesting medium (Gadd & Loos, 1941*b*).

Departures. The number of worms which left after egg laying has been expressed as a percentage of the observed entries. The most striking observation is that relating to the examination of *Desmodium* roots after 3 weeks, when 22 worms, or 61%, had left the roots after egg laying. The majority of them left after the first week, as at that time the known departures amounted to only 2%. Other notable departures occurred from turnip roots in the third week (14%) and from *Crotalaria* roots in the fifth week (12%).

If some eelworms left roots after egg laying it is possible that others left, perhaps earlier, without laying eggs. Such departures, however, should be reflected in the percentage infestations observed. For instance, at the end of 1 week the observed infestation of turnip roots was 58%, whereas at the end of the third week it was only 41%. In another experiment using 120 female *Anguillulina pratensis* and a similar technique, 68% entered turnip roots within 24 hr. The decrease in percentage infestation observed as the time before examination is increased suggests that some worms leave turnip roots without laying eggs.

By applying the χ^2 test to the observations made from turnip roots it becomes evident that the observed decreased infestation after 7 days is not statistically significant ($P = 0{\cdot}2$ approx.) whereas after 21 days it is $P < 0{\cdot}001$. When the results after 21 days are compared with those after 7 days the difference is of probable significance (P lies between 0·05 and 0·02).

There is also an apparent decrease in the observed percentage of infested *Crotalaria* roots at the examinations made after the second week. When the combined observations made at the end of the first and second weeks are compared with those made at the three later examinations a χ^2 value of 13·5 is obtained, giving a P value of less than 0·001. It is

evident, therefore, that the decrease of observed infestation of *Crotalaria* roots after the second week is of statistical significance.

This evidence, although indirect, clearly shows that an appreciable number of female worms leave turnip and *Crotalaria* roots after the second week without laying eggs. There is no evidence of a similar departure from *Tephrosia* or tea, but there is conclusive evidence of a departure from *Desmodium* after eggs have been laid.

Steiner (1934) published an illustration of a group of embryonated eggs of *Anguillulina pratensis* deposited close together within a rice root, concerning which he said: 'It is thought that a single female deposited these eggs and afterwards left, according to the vagrant habit of the species.' Vagrancy of *A. pratensis*, however, seems to be determined by the species of root into which the worms enter. In *Tephrosia* and tea roots, the females remain with their eggs and offspring forming nests of increasing size, but they have a marked tendency to depart from *Desmodium*, *Crotalaria* and turnip roots before or after laying a few eggs. Is vagrancy, then, to be regarded as a normal habit of the species, or is it imposed upon the worms by unfavourable environment?

Non-layers. The worms found within the roots at each examination have been classified as layers and non-layers of eggs. The worms which left the roots after laying a few eggs are not included in these counts. The number of non-layers at each examination has also been expressed as a percentage of the observed entries.

The results obtained from *Tephrosia* roots show no marked tendency for the percentage number of non-layers to decrease with time. It would appear that in a collection of female *Anguillulina pratensis* taken from tea roots a certain percentage is sterile, either because they have not been fertilized or because they have completed their egg-laying cycle or for other reasons. By expressing all the non-layers found in *Tephrosia* roots as a percentage of the observed entries an estimate of 18% is obtained.

The percentage non-layers found in tea and *Desmodium* roots at both examinations agree closely with the estimate obtained from *Tephrosia* roots. The percentages obtained from turnip roots are consistently higher, and that from *Crotalaria* roots at the end of the first week is the highest observed. These roots, *Crotalaria* and turnip, are also the roots from which the greatest numbers of females left without laying eggs. If the non-layers which left the roots before examination could also be included in these estimates, the effect would be to increase the percentage of non-layers above the values given in Table 1 at the examinations at 3 weeks and later.

An abnormally high percentage of non-layers may be interpreted as evidence of the operation of a factor which delays or prevents egg laying. This factor is not necessarily the same as that which stimulates or prompts the eelworms to leave the roots. In *Crotalaria* roots, egg laying by the majority of worms is delayed till the second week, in turnip roots the percentage of non-layers after the third week remains high. From these roots the departing worms, as might be expected, are mainly non-layers. The large number of worms which leave *Desmodium* roots, however, deposit eggs before leaving; so that, whatever causes them to leave does not also have the effect of delaying egg laying.

Number of eggs laid. In determining the number of eggs laid in a given time, only those roots which contain both the worm and the eggs laid by her are taken into consideration. Roots containing eggs alone are excluded from the egg count, and non-layers are excluded from the worm count before determining the average. During examinations at the end of

the third week, a number of recently hatched larvae were seen and for the purpose of estimating the number of eggs laid such larvae were counted as eggs. They were also counted separately for determining the number of eggs hatched. One *Desmodium* root at the examination made at the end of the third week contained a dead female with three recently hatched larvae and one egg in its immediate vicinity. Although that worm is shown amongst the 'layers present' neither it nor its offspring was included in the estimation of the mean number of eggs laid. The egg laying of *Anguillulina pratensis* in *Tephrosia* roots has been discussed elsewhere (Gadd & Loos, 1941*a*) and it is sufficient to note here the increasing number of eggs found at successive examinations till the end of the fourth week. During the first week, the average number deposited per egg layer was 6·4 which increased to 19·1 by the end of the third week.

In tea and *Desmodium* roots, egg laying was carried on at a similar rate during the first week, but in *Crotalaria* and turnip, egg laying was much slower. In turnip, egg laying apparently ceased after the first week when a mean of only 2·8 eggs per layer had been reached, a value less than half that observed in *Tephrosia* roots in the same time. In *Crotalaria* roots, a mean of only 9·3 eggs per layer was reached after 4 weeks, and in *Desmodium* roots only 9 eggs per layer were found at the end of 3 weeks as compared with 26 and 19 for tea and *Tephrosia* respectively. During the second and third weeks many females left *Desmodium* roots after laying eggs, but such departures can have no effect on the mean number of eggs here discussed, except in so far as they reduce the number of observations from which the mean is calculated.

If, however, larvae vacated the roots shortly after emerging from the eggs their absence would have the effect of depressing the estimate of eggs laid, as such vagrant larvae cannot be counted. The probability of larvae leaving certain roots will be discussed later. Here it is assumed that all the eggs laid during the first week in *Desmodium* roots had hatched by the end of the third week (cf. 61 % in *Tephrosia* roots): 50 % of them were found, so 50 % may have escaped. By including these hypothetical vagrants in the egg count the mean number of eggs laid per fertile worm becomes 11·5 which is still significantly smaller than the number found in tea and *Tephrosia* at that time. In the same way it may be shown that the mean number of eggs laid in *Crotalaria* and turnip roots are truly less than the numbers found in tea and *Tephrosia*.

It is therefore evident that for some reason, probably associated with environment, egg laying by *Anguillulina pratensis* in *Desmodium*, turnip and *Crotalaria* roots is markedly less than that observed in *Tephrosia* and tea roots, though the decrease in *Desmodium* roots is not observed till after the first week.

Larvae. In *Tephrosia* roots the eggs of *Anguillulina pratensis* take from 15 to 17 days to hatch. All eggs laid within 4 days after entry into a root will therefore have hatched by the end of the third week, and at that examination will be found as larvae. By the end of the fourth week all eggs laid during the first week together with some laid in the second week should occur as larvae. In Table 1 the larvae found at each examination are shown as a mean of the layers present; the same observations were excluded from these data as were excluded when calculating the mean number of eggs, viz. non-layers and eggs and larvae left by vagrant worms. There should therefore be a fairly close relationship between the mean number of larvae found at any examination and the mean number of eggs found a fortnight earlier.

At the examination made after 3 weeks, larvae were found in the roots of all plants utilized. When the mean number of larvae found at that examination is expressed as a percentage of the mean number of eggs found a fortnight earlier, it is evident that at least 50% of the eggs deposited in *Tephrosia*, tea and *Desmodium* roots during the first week had hatched; but the percentages of hatched eggs in turnip and *Crotalaria* roots were only 25 and 7 respectively.

A decrease in the observed percentage of eggs hatched at the end of the third week may be brought about in several ways. It may result from (1) a delay in egg laying till towards the end of the first week; (2) a prolongation of the incubation period; (3) a failure of many eggs to hatch, or (4) a departure of the larvae after hatching.

As in other respects the behaviour of *A. pratensis* in turnip roots is similar to that in *Crotalaria* roots, further study of hatching was restricted to *Crotalaria* roots only. If the low percentage of hatched eggs observed in *Crotalaria* roots at the end of 3 weeks was due only to a delay in egg laying during the first week, all that were laid in the first week should have hatched by the end of the fourth or fifth week; but the mean number of eggs hatched at the end of the fifth week was only 0·39, whereas an average of 2·5 eggs was deposited by each layer at the end of the first week. Nor can these figures be satisfactorily explained on the hypothesis of a prolonged incubation period. The increases in the mean number of hatched eggs observed at the end of the fourth and fifth weeks are not of statistical significance, so that it is not certain that more larvae were present at the end of the fifth week than at the end of the third. Failure of the larvae to increase in number between the third and fifth weeks may be due either to the eggs ceasing to hatch or to the larvae escaping from the roots soon after they emerged from the egg.

It was not possible in these experiments to determine which of these explanations is correct. If the figures are accepted at their face value then both egg laying and hatching must have ceased in *Crotalaria* roots at the end of the third week. If, however, the escape of larvae from the roots is assumed, the mean numbers of eggs laid at the end of the third, fourth and fifth weeks must be greater than those given in Table 1, as escaped larvae were not included in the count. Further, if the escape of larvae affords a true explanation of the small number of larvae observed, the rate of escape of larvae was practically equal to the rate of egg laying.

Growth of larvae. Seedling roots of *Tephrosia* and *Crotalaria* were infested with larvae of *Anguillulina pratensis* recently hatched in sand. The mean length measurement of the 22 worms in *Tephrosia* roots after 18 days was 454 μ whereas in *Crotalaria* roots the mean of 36 was only 316 μ: 50% of the *Crotalaria* roots contained no worm after 18 days.

In *Tephrosia* roots, larvae become adult after 15–16 days and egg laying begins about 15 days later. Eggs are found in roots examined 5 weeks after being invaded by freshly hatched larvae. No eggs were found in *Crotalaria* roots after 6 weeks. Very few worms, however, were seen at that examination; they were so few that a continuation of the experiment appeared unprofitable.

These experiments demonstrate that newly hatched larvae will invade *Crotalaria* roots, but like adults they tend to leave after a short time. Those that stay increase in size, but at a less rapid rate than when *Tephrosia* is the host plant. The experiments also suggest that the small numbers of larvae found at the later examinations of the previous experiment are not entirely due to the departure of larvae after hatching, but to a large extent to the failure of eggs to hatch.

Discussion

The host list of *Anguillulina pratensis* is already formidable and justifies Cobb's warning (1917) 'that we need not be surprised to find it infesting almost any of our crop plants'. The nematode, however, is not likely to prove a serious pest of all plants in the roots of which it may be found. Cobb (1917) states that no definite evidence exists that *A. pratensis* is a serious pest of the cotton plant; at the time he discovered it in cotton roots he had no suspicion that the roots were infested.

Whether a plant will or will not be seriously damaged directly by this nematode depends to a large extent upon the behaviour of the eelworm after entry. The worms may settle down and with their offspring form large nests as in tea and *Tephrosia* roots, or they may not find the environment suitable for breeding, with the result that they leave the roots before or after laying a few eggs; e.g. *Desmodium*, *Crotalaria* and turnip. That Steiner (1934) should regard this species as vagrant in habit suggests that in his experience *Anguillulina pratensis* is frequently met with in roots which do not form really suitable media for breeding purposes. The writers are of the opinion that the normal habit of *A. pratensis* is sedentary and that vagrancy is forced upon it when the environment in which it finds itself is unsuitable for breeding purposes.

The attractiveness of roots to *A. pratensis* is no criterion of their suitability for breeding. It has been shown (Gadd & Loos, 1941*b*) that the roots of *Crotalaria anagyroides* are as attractive to this nematode as are the roots of *Tephrosia Vogelii* but, as already shown, the later behaviour of the worms within these roots is very different. It seems probable, therefore, that by infestation experiments or by a search of roots of different species of plants growing in infested soil, the host list of this eelworm could be greatly extended. Such an extension of the host list would be of doubtful economic value unless the list also showed which species formed suitable environments for the multiplication of the worms.

Barrons (1939) working with *Heterodera marioni* showed that as many larvae entered the rootlets of Alabama No. 1, which is highly resistant, as of Kentucky Wonder bean, which is susceptible to rootknot. Also, he found from a study of the rootlets of a number of susceptible and resistant varieties and species of plants, that there were no significant differences in the rate of larval entry either in the seedling or adult stage. Any assumption that resistant plants actually resist the entry of nematode larvae is consequently untrue. Barrons proposed the hypothesis that resistance to rootknot is due to substances synthesized by the plant that counteract the giant-cell-inducing effect of the salivary secretions of *H. marioni* larvae.

Resistance to *Anguillulina pratensis* is also not due to actual resistance by the roots to entry of the nematodes, as all species here experimented with were entered by both adults and larvae. Tea was known to be susceptible, but *Desmodium gyroides*, from experience of its growth in infested soil, was considered to be resistant. The resistance of *Desmodium* is apparently due to the fact that after entry into the roots the nematodes find their environment unsuitable, and consequently leave though not before laying a few eggs, which duly hatch. On this basis, *Crotalaria anagyroides* and turnip should prove even more resistant, as more worms leave the roots before laying eggs, and when eggs are laid, they are very few. The eggs laid in *Crotalaria* either do not hatch or the larvae escape from the roots after emerging from the egg. *Tephrosia Vogelii*, however, should prove as susceptible as tea.

No specific reason is offered here for the departure of the worms after entry into certain roots. The formation of giant cells is not induced by *Anguillulina pratensis*, so that Barrons' hypothesis is not directly applicable. The fact that the main departure of worms occurs after the first week may indicate the synthesis of some substance by the plant which induces the worms to leave. On the other hand, the hypothesis of a synthesized substance is not essential to explain the observations; the departure may result directly from the unchanged chemical composition of the root juices. The new environment of *Crotalaria* and turnip roots appears to have immediate effect on the female worms which enter them in reducing the egg laying ability of the worms, and later in preventing the hatching of the eggs. These effects are more likely to be the direct result of the chemical composition of the root juices than of some substance synthesized for that purpose.

Summary

The behaviour of adult female *Anguillulina pratensis* within seedling roots of *Crotalaria anagyroides*, *Desmodium gyroides*, turnip and tea has been studied and compared with its behaviour in *Tephrosia Vogelii*. In tea roots as in *Tephrosia*, the worms settle down to a sedentary life; an average of 19 or more eggs are laid in 3 weeks and these hatch normally. In *Desmodium* roots, egg laying proceeds normally during the first week, but later diminishes. An average of 9 eggs only was laid in 3 weeks. Many worms leave the roots after laying eggs. In *Crotalaria* and turnip roots few eggs are laid and many worms leave without laying eggs. Few eggs hatch within *Crotalaria* roots or the larvae leave soon after emergence from the eggs. Resistance of roots to attack by *Anguillulina pratensis* is not a resistance to entry. It is demonstrated by the departure of worms from invaded roots and an inability of the worms to reproduce at a normal rate.

REFERENCES

Barrons, K. C. (1939). Studies of the nature of rootknot resistance. *J. agric. Res.* **58**, 263.

Cobb, N. A. (1917). A new parasitic nema found infesting cotton and potatoes. *J. agric. Res.* **11**, 27.

Gadd, C. H. & Loos, C. A. (1941*a*). Observations on the life history of *Anguillulina pratensis*. *Ann. appl. Biol.* **28**, 39.

—— —— (1941*b*). Host specialization of *Anguillulina pratensis*. I. Attractiveness of roots. *Ann. appl. Biol.* **28**, 372.

Steiner, G. (1934). Rootknot and other nematodes attacking rice and some associated weeds. *Phytopathology*, **14**, 916.

(*Received* 16 *April* 1941)

STUDIES ON THE MECHANISM OF FUNGICIDAL ACTION

I. PRELIMINARY INVESTIGATION OF NICKEL, COPPER, ZINC, SILVER AND MERCURY

By A. F. PARKER RHODES, B.A.

Agricultural and Horticultural Research Station, Long Ashton

I. Introduction

The object of this investigation was to throw light on the nature of the action of metals, and particularly copper, in inhibiting the germination of fungal spores, with a view to the better understanding of the action of copper fungicides, and in the hope of showing a way towards their improvement. To this end various compounds of nickel, copper, zinc, silver and mercury were used and their effects observed on *Macrosporium sarcinaeforme* and *Botrytis allii*. Copper, mercury and to a less extent zinc, have been used as fungicides in practice, and much work has been done on the various effects of different copper compounds as fungicides. It had been generally supposed that the effective agent in killing fungi is the Cu^{++} ion, but recently evidence has been forthcoming that this is not necessarily the case (see Horsfall *et al.* 1937) and this paper tends further to support the view that a complex ion may be the actual agent of the effect in nature. Certain regularities are also brought out in comparing the behaviour of different fungi and metals.

II. Method of approach

Theorems on the composition of variability

The term 'variability' is used here to mean a statistic involving the second but no higher moment of the variate (tolerance of spores to the action of metallic ions) which consists additively of two parts, one of which, the *fixed component*, is the same for all compounds of a given metal, while the other, the *characteristic component*, can have different values for different compounds, including zero for some particular compound or class of compounds.

The interpretation of the observational results obtained is based on two theorems:

(i) If the tolerance (measured by the atomic concentration of metal just sufficient to prevent germination of the given spore) of spores of a certain species of fungus towards compounds of a certain metal is normally distributed, then the variability can be measured by the relative variance, that is, the ratio of the variance to the square of the mean.

(ii) If the logarithm of the tolerance is normally distributed, then the variability can be measured by the variance of the logarithm.

Proofs of these theorems are offered below.

Case of normal distribution of the tolerance

Consider a population of spores germinating in a solution containing metallic ions. It is known from the work of McCallan & Wilcoxon (1930, 1936) that the spores excrete various substances into the medium. This excretion will differ from one spore to another, and will

thus bring about differences in the nature of the medium immediately adjacent to the several spores, but the body of the solution will be affected by the excretion of the population as a whole.

The metallic compound* MA, will react with the excretion in the body of the solution thus: $MA+E_1 \rightleftharpoons MB$. When the compound MB comes within the region immediately adjacent to a spore, it will react again, thus: $MB+E_2 \rightleftharpoons MC$; it is immaterial by how many steps the reaction proceeds or what is the chemical nature of the excreted mixtures denoted by E_1 and E_2. If by MC we denote the compound which is actually absorbed by the spore, we may complete the scheme by writing $MC+E_3 \rightleftharpoons MD$ for the reactions taking place within the spore, and giving rise to the actual inactivating agent MD. We must recognize however that MD and MC may be identical.

Applying the law of mass action to these reactions, we have

$$\frac{[MA].[E_1]}{[MB]}=K_1$$

and

$$\frac{[MB].[E_2]}{[MC]}=K_2$$

and

$$\frac{[MC].[E_3]}{[MD]}=K_3.$$

Let us now put $K_1/[E_1]=J$; $K_2/[E_2]=K$; $K_3[MD]/[E_3]=L$; then

$$[MA]=JKL. \tag{1}$$

Now the value of J depends upon the reactions taking place in the body of the solution, and consequently its value is the same for every spore, though it may be different for different compounds. The value of K depends on reactions taking place at the surface of the spore, and so may differ from one spore to another; nor is there any reason to assume that it will be independent of the nature of MA and MB. But it is reasonable to suppose that for a given species of fungus, the compound MC resulting from this reaction is always the same, independently of MA, so that the value of L will depend only on the peculiarities of each individual spore.

If $[MA]$ be considered as the tolerance (as defined above) of a given spore, it follows that only K and L will contribute to its variance (it is assumed that there is no correlation between K and L), i.e.

$$\begin{aligned} V([MA]) &= \bar{J}^2 . V(KL) \\ &= \bar{J}^2 \{\bar{K}^2 V(L)+\bar{L}^2 V(K)+V(K)\,V(L)\}, \end{aligned} \tag{2}$$

$$\therefore \frac{V([MA])}{[MA]^2}=\frac{J^2}{\bar{J}^2\bar{K}^2\bar{L}^2}\{\bar{K}^2 V(L)+\bar{L}^2 V(K)+V(K)\,V(L)\}$$

$$=\frac{V(L)}{\bar{L}^2}+\frac{V(K)}{\bar{K}^2}\left(1+\frac{V(L)}{\bar{L}^2}\right),$$

which we can write with new symbols as

$$u=u_0+u_1(1+u_0). \tag{3}$$

Since L (and therefore $V(L)$) is independent of the original compound MA or MB whereas

* If at the dilutions required electrolytes are completely dissociated, the argument applies to ions no less than to neutral molecules; evidence that this is in fact the case appears later in this paper.

K is not so, it is clear that the quantity u (*relative variance* of the tolerance) is composed additively of two parts, of which only one is affected by the nature of the compound, and furthermore if $[MB]=[MC]$, $K_2=E_2$, and $K=1$, and therefore $V(K)=0$, so that for certain compounds $u_1=0$. u therefore satisfies the definition of 'variability'.

Case of normal distribution of logarithmic tolerance

The argument is identical up to the equation

$$[MA]=JKL. \tag{1}$$

Here, however, for the variance we must write

$$V(\log [MA])=V(\log KL)$$
$$=V(\log K)+V(\log L)$$

(provided $\log K$ and $\log L$ are uncorrelated), which we will rewrite as

$$v=v_0+v_1. \tag{4}$$

Applying the same considerations as above, it follows that the quantity v (variance of the logarithm of the tolerance) satisfies the conditions set out in the definition of 'variability'.

Use of the above theorems in interpretation of the data

By arguments similar to those set out above it can be shown that if the reaction $MB+E_2 \rightleftharpoons MC$ proceeds by a number of stages, each stage will contribute separately to the variability v_1 or u_1. So that if we can arrange a series of compounds of a given metal in order of the variability of the tolerance of the test spores to them, measured by the appropriate statistic, and if we demonstrate a significant difference between two of them, it may be that the one showing the smaller variability is converted in nature into the form MC by a smaller number of stages than the other. On the other hand it can be shown that various other factors can affect the variability, notably the permeability of the spore wall, the stability of the compound MB, and the velocities of the various reactions taking place. Attention must therefore be given to the chemical aspect of each case.

III. Experimental technique

(1) *Biological materials*

The biological materials used were cultures of *Macrosporium sarcinaeforme* and *Botrytis allii*. The *Macrosporium* culture was derived from an original collection which had been taken on clover. The *Botrytis* collection was from onions in Lincolnshire. The cultures were slopes on malt extract agar. The *Macrosporium* was subcultured at intervals of not more than 14 days; the *Botrytis* usually at weekly intervals. The slopes were kept in an incubator adjusted to 21° C. Unfortunately, owing to an interruption in the gas supply the temperature was subject to rather wide fluctuations during part of the period of these experiments, and this appeared to affect the response of the fungi in the fungicidal tests. The nature of the irregularities observed is commented on later (see p. 403).

(2) *Chemical materials*

As a source of the ordinary ions of the metals studied, Cu^{++}, Zn^{++}, Ni^{++}, Ag^{+}, and Hg^{++} 'analar' reagents were used, namely cupric sulphate, zinc sulphate, zinc chloride, nickel sulphate, silver nitrate, mercuric chloride and acetate. In the cases of copper and zinc insoluble compounds were also used, with the former Bordeaux mixture, cuprous oxides (red and yellow) and mono-

thioureocuprous chloride, and with the latter, zinc oxide. For non-thioureous complex ions of copper, cupric malonate,* and the copper compounds of glycine, alanine, and *d*-valine* were used. The preparation of thiourea complexes of the metals was carried out by the author, following in general the methods of Rosenheim *et al.*:

(1) *Trithioureocuprous chloride* was prepared from its constituents; the recrystallized preparation contained 19·73% Cu (determined by ignition to CuO). Theoretically $Cu_3SC(NH_2)_2Cl$ contains 19·43% Cu (Rosenheim & Loewenstamm, 1903).

(2) *Monothioureocuprous chloride* by adding excess of hydrochloric acid to the above; it contained 34·60% Cu, as against the theoretical 34·57% for $CuSC(NH_2)_2Cl$. This compound is insoluble in water, cold or hot (Rosenheim & Loewenstamm, 1903).

(3) *Pentathioureocuprous sulphate* from the hydroxide and sulphuric acid. It contained 19·84% Cu, as against the theoretical 19·78% for $Cu_25SC(NH_2)2H_2O.SO_4$. Although stated by Rosenheim & Loewenstamm (1903) to be unstable, the aqueous solution did not decompose appreciably in the absence of alkali.

(4) *Trithioureozinc sulphate* from its constituents. The content of zinc was 17·67% as against 16·80% for $Zn_3SC(NH_2)_2SO_4$ (Rosenheim & Meyer, 1906).

(5) *Dithioureozinc chloride* from its constituents. It contained 22·87% Zn as against 22·67% Zn for $Zn_2SC(NH_2)_2.Cl_2$. Zinc determination was by precipitation as carbonate and igniting to the oxide (for reference see Beilstein, 1893).

(6) *Dithioureargentous chloride* from its constituents. The silver content, determined by ignition to the metal, was 35·8% Ag, as against 36·5% Ag for $Ag.2SC(NH_2)_2.Cl$ (Beilstein, 1893).

(7) *Dithioureargentous nitrate* from its constituents. The salt was recrystallized from dilute nitric acid; it took the form of fine white needle-shaped crystals, soluble in water, sparingly soluble in dilute nitric acid; the aqueous solution slowly decomposed forming a silver mirror on the walls of the vessel. The silver content was 33·8% Ag, as against 34·8% Ag for $Ag2SC(NH_2)_2NO_3$.

(8) *Tetrathioureomercuric chloride* from its constituents (for details see Rosenheim & Meyer, 1906).

Certain experiments were performed in which the active solution contained a mixture of a single salt of one of the above metals and thiourea, the former only being varied in concentration. Except in one instance with zinc sulphate, and regularly with nickel sulphate, such mixtures behaved towards the spores in the same way as the crystalline compounds described above, except that alterations usually took place in the quantities of metal required to produce a given effect. As these effects are clearly due to the formation of the complex ion in solution, the figures for such mixtures were combined with those for the crystalline compounds in presenting the results.

Nickel was included among the metals used as a check on the others; according to Rosenheim & Meyer (1906) the thiourea complexes of this metal, though existing in alcoholic solution, are completely hydrolysed in the presence of water.

(3) *Methods of dealing with insoluble compounds*

In determining the reaction of the experimental organisms towards insoluble compounds of the metals used, the latter were sprayed on to prepared slides in a standard manner and the spores brought into contact with the deposit in drops of water of measured area and volume.

Suspensions of Bordeaux mixture were prepared by mixing equal volumes (normally 100 c.c.) of a solution of cupric sulphate and a suspension of ground calcium hydroxide, analytically free from carbonate. The solution was such as to contain the same weight of $CuSO_4.5H_2O$ as that of $Ca(OH)_2$ contained in the suspension, the actual quantities being based on the concentration of suspension required. The solution was added quickly to the suspension, and the mixture shaken thoroughly and sprayed within 4 hr.

Spraying was carried out with a horizontal atomizer of the kind described by Evans & Martin (1935), the air pressure applied being 39 cm. of mercury and the duration of delivery 10 sec. Under these conditions the apparatus used would deliver 0·00606 g. of distilled water/sq. cm. of slide surface, averaged over the area of an ordinary microscope slide. Between spraying different compounds, the atomizer was washed with distilled water. The slides on to which suspensions were sprayed were prepared by immersion in a solution, containing 25 g. cellulose nitrate in 1 litre butyl acetate, followed by drying at a slight inclination.

Dry materials brought into suspension for spraying had to be treated with care to ensure a uniform

* For the behaviour of these compounds as complex ions, see Riley (1930) and Riley & Gallafent (1931).

and reasonably stable suspension. The material first had to be reduced in the dry state to an impalpable powder, and in the suspending of it had to be triturated under water until decantation left a negligible residue.

(4) *Methods of dealing with soluble compounds*

The usual procedure was to prepare a series of solutions each having a concentration twice that required for investigation. Drops of these solutions, 0·02 c.c. in volume, were deposited on slides prepared as for spraying (usually 5 drops per slide), and each mixed with 0·02 c.c. of a suspension of the spores to be tested, so that the volume of each final drop was 0·04 c.c. Such a drop on the slides prepared as described, if deposited with care, occupied about 0·38 sq. cm. (on this area there would be 0·0023 g. of sprayed suspension, so that a concentration of suspension x would give, supposing all the solid dissolved, a concentration in the drop of $0{\cdot}0573x$).

Concentrations were always expressed on an arbitrary logarithmic scale, with base 2 and unit $= 6{\cdot}77 \times 10^{-6}$ g.-atoms of metal/l.

(5) *Preparation of spore suspensions*

A slope culture of the fungus to be used, of suitable age, was flooded with about 2 c.c. of distilled water. The surface of the culture was gently rubbed with a glass rod to detach and facilitate the wetting of the spores; the crude suspension thus obtained, containing some of the culture medium, was freed of visible pieces of agar or mycelium with forceps and three times washed with distilled water by centrifugation, using a small hand centrifuge. The washed suspension was then diluted in a watch glass to a suitable spore density, this being judged by eye. In depositing drops of suspension on the slides, the liquid was agitated by bubbling air through it from the micro-pipette, thus ensuring good uniformity of density throughout any one experiment.

In some cases, particularly when the variance of tolerance was small, there was a noticeable difference in percentage germination between the more crowded and less crowded parts of one drop, due presumably to competition between adjacent spores for the available metallic ions. For this reason less concentrated suspensions were favoured, the limiting factor being the need to have an adequate number of spores in each drop. So long as the average volume of solution available to each spore is greater than the mean volume from which diffusion can take place to the spore within the period of the experiment, this effect did not occur, and the actual spore density appeared to be immaterial.

(6) *Technique of spore counting*

After an appropriate period (48 hr. was in all cases sufficient) the germination was counted. For this purpose the slides (which had been kept meanwhile in a fairly uniform room-temperature incubator, in a saturated atmosphere) were inverted on two glass rings on the microscope stage. Each drop was then examined in turn under a suitable magnification, and in each 50 or 100 spores were counted, those having germinated being registered on a hand counter.

TABLE 1. *Exp.* 2662: *Effect of cupric sulphate on* B. allii. *Spores from culture started* 8. i. 41; *test made* 20. i. 41

Slide no.	2	3	4	5	6	7	8	9
Concentration on arb. log. scale	0	−1	−2	−3	−4	−5	−6	$-\infty$
% germination in drops	8	7	3	17	73	85	95	96
	3	2	9	28	66	—	87	95 97
	4	5	9	22	65	85	—	98 98
	1	6	11	19	67	—	96	94 99
	1	3	11	24	65	82	97	99
Mean	3·4	4·6	8·6	22·0	67·2	84·0	93·75	97·0
q (see § IV)	0·9649	0·9526	0·9114	0·773	0·307	0·134	0·034	0

In some cases, difficulty was experienced in deciding what to accept as a criterion of germination, and this sometimes affected the results to a certain extent. In the case of *M. sarcinaeforme*, the most consistent results were obtained if spores showing bulbous or irregular germ tubes less than the spore diameter were reckoned as being ungerminated, whereas with *B. allii*, even a very small germ

tube was allowed to denote germination. The spore suspensions always contained immature spores and mycelial fragments; the germinative powers of these commonly differed from those of normal spores, and they were in all cases neglected in counting, as were also abnormally large and abnormally small spores.

The results obtained were tabulated in the form shown (in Table 1), which is an actual specimen from the experimenter's note-book.

IV. Statistical methods

Computation of primary statistics

From the recorded data from tables of the type of Table 1 (which are brought together in Tables 2–12) the statistics $\bar{x}$ (mean logarithmic dosage), $\bar{y}$ (mean probit mortality) and b (regression of y against x) were computed according to the method of Bliss (1935*a*). Where applicable the mean tolerance* m was also calculated from the formula $m=\bar{x}+\frac{5-\bar{y}}{b}$; values of m are given in Table 13.

The following points require mention: (*a*) The proportional mortality q is equal to unity less the ratio of the percentage germination of the given concentration to that in distilled water controls. No experiment was admitted where less than 200 spores had been counted in the controls. Usually there were 500 or 1000 control spores. (*b*) The number of spores counted was divided by 50 in calculating the weight of the observations, since the spores in one drop are not properly independent observations; but on the other hand a drop in which 100 spores is counted (as with *Botrytis*) is clearly a weightier sample than one with only fifty spores (as with *Macrosporium*).

Computation of variability

Where, as determined graphically (checked where necessary by means of the χ^2-test) the logarithmic dosage bore a linear relation to probit mortality, the variability was given (according to § II, para. 3) by the variance v. Bliss's probits are simply the abscissae read off on the curve of the probability integral from the ordinates which are taken to represent the proportional mortality q, the integral being calculated with zero mean and unit standard deviation. Hence, representing probits by y, and the normal variate by x,

$$\frac{dy}{dw}=1, \text{ where } w=\frac{x-\bar{x}}{\sigma},$$

$$\therefore \frac{dy}{dx}=\frac{dy}{dw}.\frac{dw}{dx}=\frac{1}{\sigma}=b,$$

$$\therefore \quad v=\frac{1}{b^2}.$$

Where the dosage is itself linearly related to the probit mortality, the variability is equal to $u=v/m^2$. This was calculated from $u=1/b'^2$, b' being the repression of z against y, where $z=2^x/m$, m being estimated from $\overline{2^x}$ and $\bar{y}$ by the above formula (m was expressed, of course, not on the logarithmic scale); for in this case $b'=\frac{dy}{dz}=\frac{dy}{d2^x}.\frac{d2^x}{dz}=m\frac{dy}{d2^x}$ but since 2^x is the normal variate, $\frac{dy}{d2^x}=\frac{1}{\sigma}$ by the argument above, hence $b'=\frac{m}{\sigma}$ and $u=\frac{\sigma^2}{m^2}=\frac{1}{b'^2}$.

Tests of significance applied

Homogeneity was tested by the χ^2-test; where the data were homogeneous, or could be made so as described below, χ^2-tests could also be applied to test significance in differences between m and b (or b') values as described by Bliss (1935*b*). In cases of an irreducible heterogeneity, it would be necessary to resort to the less accurate t-test.

* This is the same quantity as 'LD 50' of many authors.

TABLE 2. Macrosporium. *Complex cupric ions*

Malonate, 3 exps.			Glycinate, 3 exps.			Alaninate, 1 exp.			*d*-Valinate, 1 exp.		
x	*q*	*v*	*x*	*q*	*v*	*x*	*q*	*v*	*x*	*q*	*v*
0·70	0·177	5	2·00	0·393	5	2·21	0·190	10	4·53	0·537	10
1·70	0·255	5	2·21	0·105	5	3·21	0·283	9	5·53	0·770	10
2·21	0·285	10	3·00	0·492	5	3·80	0·401	10	6·18	0·835	10
2·70	0·387	5	3·80	0·482	9	4·21	0·465	9	6·53	0·917	9
3·21	0·645	10	4·00	0·570	5	4·53	0·577	10	6·85	0·931	7
3·70	0·618	10	4·21	0·583	8						
3·80	0·886	9	4·53	0·658	9						
4·21	0·938	10	5·00	0·514	5						
4·53	0·956	7	6·00	0·718	10						
4·70	0·785	5	7·00	0·839	10						
5·70	0·838	8	8·00	0·850	9						
6·70	0·898	10									

TABLE 3. Macrosporium. *Compounds producing simple cupric ions*

Bordeaux mixture, 5 exps.			Cupric sulphate, 3 exps.			Cuprous oxide, 4 exps.		
x	*q*	*v*	*x*	*q*	*v*	*x*	*q*	*v*
0·72	0·013	6	2·075	0·222	10	1·50	0·013	15
1·19	0·126	6	2·212	0·297	9	2·07	0·051	15
1·50	0·172	18	2·650	0·525	9	2·63	0·124	19
1·88	0·322	6	3·212	0·782	15	3·21	0·359	20
2·07	0·441	21	3·799	0·916	13	3·80	0·574	20
2·46	0·605	6	4·212	0·968	15	4·53	0·802	19
2·65	0·561	21	4·535	0·9967	19			
3·21	0·793	21						
3·80	0·9889	20						
4·38	0·9882	11						
4·53	1·0000	10						

TABLE 4. Macrosporium. *Compounds and mixtures producing Trithioureocuprous ions*

Trithioureocuprous chloride, 4 exps.			Monothioureocuprous chloride, 2 exps.			Pentathioureocuprous sulphate, 3 exps.			Mixtures containing thiourea,* 2 exps.		
x	*q*	*v*	*x*	*q*	*v*	*x*	*q*	*v*	*x*	*q*	*v*
1·21	0·010	5	1·50	0·085	3	1·50	0·031	15	1·50	0·020	5
1·50	0·027	10	2·075	0·079	5	2·075	0·055	15	2·075	0·037	5
1·80	0·048	5	2·33	0·043	4	2·65	0·681	17	2·15	0·001	4
1·925	0·000	3	2·59	0·220	2	3·21	0·9891	16	2·65	0·324	5
2·075	0·151	8	2·65	0·298	5	3·80	0·9988	17	2·71	0·175	5
2·21	0·070	5	2·86	0·577	4	4·53	1·0000	18	3·21	0·965	5
2·50	0·000	5	3·21	0·971	3				3·30	0·9833	5
2·65	0·692	10	3·39	0·9890	2				3·80	0·9913	5
2·80	0·875	3	3·80	0·972	4				4·53	1·0000	5
3·075	0·749	10	4·38	1·0000	4						
3·21	0·9934	15									
3·65	0·9829	5									
3·80	1·0000	15									

* In one experiment, the thiourea acted upon cuprous oxide in the presence of acid; in the other upon cupric sulphate. In the latter case, the reaction was such as to raise the mean by 0·5 on the log. scale. An appropriate correction has been applied to the figures.

TABLE 5. Macrosporium. *Effect of zinc compounds*

Dithioureozinc* chloride and sulphate, 6 exps.			Zinc chloride and sulphate, 3 exps.			Zinc oxide, 2 exps.		
x	q	ν	x	q	ν	x	q	ν
1·637	0·003	10	1·0	0·043	5	2·0	0·058	10
2·000	0·016	5	1·175	0·413	5	3·0	0·163	10
2·115	0·013	5	2·0	0·120	10	4·0	0·237	10
2·174	0·015	9	2·175	0·216	4	5·0	0·394	10
2·637	0·044	9	3·0	0·249	8	6·0	0·476	10
2·702	0·027	5	3·175	0·556	5	7·0	0·630	10
3·000	0·117	5	4·0	0·319	9	8·0	0·797	9
3·175	0·069	9	4·175	0·653	5			
3·289	0·114	4	5·0	0·594	5			
3·637	0·158	10	5·175	0·744	5			
3·876	0·391	5	6·0	0·814	5			
4·000	0·576	5	6·175	0·612	5			
4·175	0·477	10	7·0	0·661	5			
4·463	0·914	5	7·175	0·861	5			
4·637	0·650	10	8·175	0·770	5			
5·000	0·907	5	9·175	0·908	5			
5·050	0·955	5						
5·175	0·937	10						
5·637	0·975	13						
6·000	0·9912	5						
6·637	0·970	5						
7·000	1·0000	4						
7·175	0·9958	10						
8·175	1·0000	5						

* Including one experiment on a mixture containing thiourea and zinc sulphate.

TABLE 6. Macrosporium. *Effect of silver compounds*

Dithioureargentous chloride and nitrate, 3 exps.				Silver nitrate, 2 exps.			
x	z*	q	ν	x	z	q	ν
0·02	0·232	0·000	4	0·02	0·175	0·006	4
0·92	0·454	0·086	5	1·02	0·350	0·019	9
1·02	0·473	0·552	5	2·02	0·700	0·138	9
1·74	0·848	0·642	5	3·02	1·400	0·378	10
2·02	0·942	0·330	10	4·02	2·800	1·000	9
2·65	1·632	0·126	5				
2·74	1·695	0·680	5				
3·02	1·884	0·773	6				
3·80	3·474	0·611	4				

* For the calculation of z see text (p. 394). The values of m are as follows: Dithioureargentous compounds $m=4·29$, Silver nitrate $m=5·53$.

TABLE 7. Macrosporium. *Effect of mercurials*

Mercuric chloride and acetate, 4 exps.			Tetrathioureomercuric chloride, 1 exp.		
x	q	ν	x	q	ν
−2·76	0·014	10	−2·45	0·026	5
−2·00	0·014	5	−1·45	0·071	5
−1·76	0·106	10	−0·45	0·255	5
−1·00	0·117	4	1·55	0·202	5
−0·76	0·090	15	2·55	0·360	4
0	0·319	5	3·55	0·505	5
0·24	0·332	15			
1·00	0·598	3			
1·24	0·701	9			
2·00	0·856	5			
2·24	0·922	13			
3·24	0·9909	9			
4·24	0·9949	4			
5·24	1·0000	5			

TABLE 8. Macrosporium. *Effect of nickel*

In the presence of thiourea, 2 exps.			In the absence of thiourea, 2 exps.		
x	q	v	x	q	v
−3·0	0·011	3	−2·0	0·011	5
−2·0	−0·008*	5	−1·0	0·050	5
−1·0	0·175	5	0	0·095	5
0	0·223	5	1·0	0·313	10
1·0	0·540	10	2·0	0·422	5
2·0	0·754	5	3·0	0·774	4
3·0	0·961	4	5·0	0·924	5
5·0	0·9934	4	7·0	0·935	5
7·0	0·9895	5	9·0	1·0000	5
9·0	1·0000	5			

* All other values of q are positive. (In this case germination exceeded that of the controls.)

TABLE 9. Botrytis. *Effect of copper compounds*

Cupric glycinate and malonate, 3 exps.				Cupric sulphate, 2 exps.				Trithioureocuprous chloride, 3 exps.			
x'	x*	q	v	x'	x	q	v	x'	x	q	v
−1	1·5	0·068	10	−6	0·8	0·034	8	−3	1·3	0·023	10
0	2·5	0·203	10	−5	1·8	0·134	6	−1	3·3	0·086	10
1	3·5	0·251	10	−4	2·8	0·307	10	0	4·3	0·910	6
2	4·5	0·392	8	−3	3·8	0·773	10	1	5·3	0·9839	4
3	5·5	0·625	4	−2	4·8	0·911	10	2	6·3	0·9919	8
4	6·5	0·769	10	−1	5·8	0·953	10				
				0	6·8	0·984	10	−1	2·6	0·015	10
−3	2·0	0·142	8					0	3·6	0·444	8
−2	3·0	0·184	18*	−3	1·0	0·052	8				
−1	4·0	0·308	20	−2	2·0	0·095	10	1·17	3·17	0·037	8
0	5·0	0·488	18	−1	3·0	0·530	10	1·58	3·58	0·370	10
1	6·0	0·767	18	0	4·0	0·813	10				
2	7·0	0·868	16								
3	8·0	0·969	18								

* In tables dealing with results obtained with *B. allii* the column headed x' gives the values of the logarithmic concentration actually applied, while under x are listed the values corrected for homogeneity as described in the text. The values of z where required are not subject to correction.

TABLE 10. Botrytis. *Effect of zinc compounds*

Zinc sulphate, 3 exps.					Dithioureozinc chloride, 2 exps.				
Mean	x'	z	q	v	Mean	x'	z	q	v
Exp. 2771	5	1·523	0·008	10	*Exp.* 2781	3	1·667	0·002	10
m=21·0	6	3·046	0·051	10	m=4·8	4	3·333	0·034	8
	7	6·092	0·311	10		5	6·667	0·451	10
	8	12·184	0·833	10		6	13·333	0·9937	10
Exp. 2772	6	2·346	0·042	8	*Exp.* 2782	5	1·361	0·011	10
m=28·0	7	4·692	0·129	8	m=23·5	6	2·722	0·042	8
	8	9·384	0·555	10		7	5·444	0·231	10
Exp. 2773	4	0·308	0·044	8		8	10·888	0·885	10
m=52·9	5	0·616	0·114	10					
	6	1·232	0·125	10					
	7	2·464	0·242	10					
	8	4·928	0·397	10					
	9	9·856	0·535	10					
	10	19·712	0·680	10					

TABLE 11. Botrytis. *Effect of silver compounds*

Dithioureargentous chloride, 2 exps.				Silver nitrate, 2 exps.			
x'	x	q	v	x'	x	q	v
−2·00	0·00	0·003	10	−2	0	0·013	8
−1·00	1·00	0·025	10	−1	1	0·175	20
0·00	2·00	0·067	8	0	2	0·767	20
−0·55	2·45	0·174	8	1	3	0·831	18
1·00	3·00	0·117	10	2	4	0·904	20
0·45	3·45	0·359	10	3	5	0·970	18
2·00	4·00	0·628	6				
1·45	4·45	0·696	10				
3·00	5·00	0·831	10				
2·45	5·45	0·971	10				
3·45	6·45	0·9941	8				
4·45	7·45	0·9976	10				

TABLE 12. Botrytis. *Effect of mercurials*

Tetrathioureomercuric chloride, 3 exps.				Mercuric acetate, 1 exp.			Mercuric chloride, 1 exp.			
x'	x	q	v	$x'=x$	q	v	x'	x	q	v
−2	0·0	0·048	20	−0·76	0·005	10	−1	1	0·033	10
−1	1·0	0·210	30	0·24	0·020	10	0	2	0·756	6
0	2·0	0·375	25	1·24	0·083	10	1	3	0·9575	8
−1	2·5	0·538	10	2·24	0·610	10	2	4	0·9933	10
1	3·0	0·560	28	3·24	0·9920	8	3	5	1·0000	10
0	3·5	0·693	10							
2	4·0	0·747	20							
1	4·5	0·776	8							
3	5·0	0·934	20							
2	5·5	0·854	10							
4	6·0	0·9802	8							
3	6·5	0·911	10							

In Tables 13 and 14 significant differences are given between adjacent comparable results, which represent the difference significant at the 5 % level calculated from the formulae:

$$d_b = \sqrt{\left(3{\cdot}84\,\frac{A_1+A_2}{A_1A_2}\right)},$$

$$d_m = \sqrt{\left(3{\cdot}84\,\frac{\Sigma\,(w)_1+\Sigma\,(w)_2}{\Sigma\,(w)_1\,\Sigma\,(w)_2}\right)},$$

using Bliss's notation; 3·84 is the value of χ^2 with 1 degree of freedom for $P=0{\cdot}05$. Since v is uniquely determined by b, and u by b', a significant difference in b or b' implies an equally significant one in the corresponding values of v or u.

Elimination of heterogeneity

In some cases the data for a given compound and a given species of fungus, though each separate experiment was always homogeneous, were heterogeneous when combined, owing to fluctuations in the mean tolerance. These fluctuations were traced to irregularities in the temperature at which the cultures were kept, owing to an interruption in the gas supply. Notwithstanding this, if a correction was applied to each experiment, multiplying each dosage by a constant factor (or making a constant addition to each logarithmic dosage), the heterogeneity could be wholly eliminated; this procedure

does not alter the value of b for any experiment, but clearly invalidates any estimate of m, and therefore cannot be applied to cases where b' must be calculated. In these cases it could be shown that there were no significant differences between the values of b' in the discrepant experiments; and since, by definition, the value of $\bar{x}$ is independent of m, no heterogeneity could arise. This can be verified from the data of Table 10.

Correction of variability to a common logarithmic scale

In Tables 13 and 14 values of u and v in terms of 10^{-6} g.-atoms/l. and (in the latter case) to the logarithmic base 10 are given in the last column. The corrections are as follows:

$$u' = u,$$
$$v' = 0{\cdot}0906v.$$

TABLE 13. *Summary of results with* Macrosporium

Nature of toxin		Statistics calculated					
		Primary statistics				Variability (v or u)	
		m		b or b'			
Metal	Compound	Value	Sig. dif.	Value	Sig. dif.	Arb. scale	Common scale
Copper (o)	Cupric glycinate	3·86*	0·421	0·295	0·308	11·5	1·044
	Cupric malonate	2·64*	0·470	0·579	0·809	2·98	0·271
	Cupric alaninate	4·16	0·565	0·719	0·945	1·93	0·175
	Cupric *d*-valinate	4·98		1·068		0·88	0·079
	Cuprous oxide	3·65†	0·400	1·06	0·538	0·889	0·0803
	Bordeaux mixture	2·33		1·10		0·828	0·0750
	Cupric sulphate	2·67	0·441	1·33	0·560	0·564	0·0512
	Combined figures for:						
	A. Complex cupric ions	—	—	0·464	0·268	4·65	0·421
	B. Simple cupric ion	2·47†	0·280	1·15		0·757	0·068
	C. Trithioureocuprous ion	2·50		2·13	0·320	0·221	0·020
Zinc (o)	Zinc oxide	5·99‡	—	0·347	0·198	8·31	0·748
	Zinc sulphate	4·52		0·208		23·04	2·047
	Combined figures for:						
	D. Simple zinc ion	4·52‡	0·44	0·269	0·296	13·8	1·25
	E. Dithioureozinc ion	4·08		1·282		0·609	0·055
Silver (1)	Combined figures for:						
	F. Silver nitrate	5·97	0·74	2·695	0·532	0·138	0·138
	G. Dithioureargentous ion	4·98		1·588		0·397	0·397
Mercury (o)	Combined figures for:						
	H. Mercuric salts	0·456	0·62	0·811	0·370	1·52	0·137
	J. Tetrathioureomercuric ion	3·755		0·270		13·7	1·241
Nickel (o)	Combined figures for:						
	Nickel sulphate with and without added thiourea	4·38	0·88	0·569	0·57	3·09	0·278

* Ignoring aberrant values obtained later with differently treated cultures.

† The fact that the value of m for cuprous oxide is approximately 1·0 greater than for cupric sulphate suggests that the cuprous oxide yields only half its copper in an available form. For this reason the combined m-value is calculated taking this figure to be 2·65.

‡ No value attaches to the mean tolerance towards zinc oxide, because of its incomplete dissolution in the germination medium.

TABLE 14. *Summary of results with* Botrytis

Nature of toxin		Statistics computed: Primary statistics, b or b'		Variability, v or u	
Metal	Compound	Value	Sig. diff.	Arb. scale	Common scale
Copper (o)	A. Complex cupric ions	0·496	0·310	4·061	0·368
	B. Simple cupric ion	0·839		1·418	0·129
	C. Trithioureocuprous ion	1·582	0·725	0·440	0·040
Zinc (1)	D. Simple zinc ion	0·168	0·182	35·9	35·9
	E. Dithioureozinc ion	0·390		6·58	6·58
Silver (o)	F. Simple silver ion	1·01	0·30	0·98	0·089
	G. Dithioureargentous ion	0·88		1·29	0·117
Mercury (o)	H. Mercuric acetate	1·63	1·18	0·376	0·034
	J. Mercuric chloride	1·98	0·87	0·255	0·023
	K. Tetrathioureomercuric ion	0·52		3·70	0·335

In Tables 13 and 14 the sign (1) indicates that the *tolerance*, and (o) that the *logarithm of the tolerance* of the spores to compounds of the metal so marked is normally distributed.

V. Presentation of results

The results are presented in Tables 2–14 which give x, the logarithmic concentration, where necessary the corresponding values of z, the proportional mortality q, and the effective number of observations v at each level of x. The number of separate experiments (each one of which was recorded after the pattern Table 1) which are combined together is also given. In Tables 9–12 a separate column is entered headed x' in which the actual logarithmic concentrations applied are recorded. As explained in the previous section, adjustments had to be applied to these values (in any case the occurrence of negative values makes more difficult the statistical computations); the *adjusted* values are given in the x column. The statistics calculated from these figures are summarized in Table 13.

VI. Discussion

Conclusions to be drawn from the results

Examination of Tables 13 and 14 shows that there is a significant difference between the variabilities of the spores towards simple ions and ions co-ordinated with thiourea, with both fungi and all the metals used except nickel (which forms no compound with thiourea in aqueous solution) and, in the case of *Botrytis allii*, silver.

With copper and zinc, the difference is such that the variability towards the thiourea complexes is less than that towards the simple ions. With silver and mercury the reverse holds. The insignificant difference observed with silver and *Botrytis* also shows greater variability for the thiourea compound. To the non-thioureous complex ions of copper, both fungi reveal a significantly greater variability than to the simple cupric ion. Among the different compounds giving rise to simple cupric or zinc ions, no differences in variability are significant, but among the four cupric complexes separately investigated with *Macrosporium*, the variability towards the glycinate is significantly less than towards the valinate.

Although not indicated in Table 13 it can be calculated from the data of Table 4 and inferred from the statistical homogeneity of the data in Tables 5, 6, 7, etc., that there is no significant difference in the variability of either species of spores to different salts of the

same cation. Thus these electrolytes, as would be expected considering the high dilutions used, behave as though completely dissociated. It is perhaps of interest that this also holds for the weakly dissociated mercuric chloride. In considering the possible changes which the metals undergo before absorption by the spores, we can therefore confine our attention to the cations only.

Inference concerning the chemical nature of the effective compounds

It was shown in § II, para. 4, that the variability of any population of spores towards a given compound was necessarily greater, the greater number of steps in the chemical reaction required to bring the compound supplied into the form in which it is absorbed by the spores; while the compound actually absorbed should give a minimum value u_0 or v_0. Consequently one possible interpretation of the results, showing a decreasing variability towards copper compounds in the order cupric complexes, simple cupric ion, thioureocuprous ion, is that these represent successive stages in the reactions undergone by the spore. In regard to the last stage, however, it has been shown by Rosenheim & Stadler (1906) that on dilution the trithioureocuprous ion undergoes a partial hydrolysis, thus:

$$[Cu3SC(NH_2)_2]^+ + H_2O \rightarrow [Cu2SC(NH_2)_2.H_2O]^+ + SC(NH_2)_2,$$

so that we must suppose the actual cation concerned to be the monohydrated dithioureocuprous ion.

We may therefore tentatively suggest (i) that such complexes as the glycinate (glycinocuprate $[Cu3NH_2CH_2COO]^-$) must first be broken down to yield simple Cu^{++} ions, and (ii) that the latter must be brought to the thioureated form thus:

$$(a)\ Cu^{++} + e \rightarrow Cu^+,$$

$$(b)\ Cu^+ + 2SC(NH)_2 + H_2O \rightarrow [Cu2SC(NH_2)_2.H_2O]^+.$$

The reduction (*a*) can in vitro be accomplished by thiourea itself, so that thioureation may be a one-stage reaction, but against this it is impossible that, even if any thiourea is available, it could suffice to reduce the copper when the latter is present in large excess. For this reason the reaction probably does not proceed in this way in Nature. (This difficulty does not arise in the case of zinc.)

It is, of course, probable that there are other causes of the difference in variability observed. But if the interpretation suggested be accepted it can be inferred that one possible effective compound is either the ion $[Cu2SC(NH_2)_2.H_2O]^+$, or some derivative of it which can be formed without destroying the co-ordinate links. It may equally well be that as well as thiourea itself other closely related compounds exist, for which thiourea is an adequate substitute; in any case we cannot draw a more definite conclusion until a much wider range of complexes has been investigated.

It is of interest to consider the cases presented by the cation $[CuSC(NH_2)_2]^+$ (see Table 5). This gives the same low value for variability with *Macrosporium* spores, yet some change must occur if the ultimate compound is necessarily $[Cu2SC(NH_2)_2.H_2O]^+$ or a derivative. In view of the ease with which monothioureocuprous chloride, insoluble in water, dissolves in dilute thiourea solution, it is reasonable to suppose that these ions could be converted to the trithioureocuprous form by thiourea (or an effective substitute) present in the body of the medium. In this case $[Cu.SC(NH_2)_2]^+$ would correspond to the compound *MA* and

$[Cu2SC(NH_2)_2H_2O]^+$ to *MB* in the terminology of § II; it is only *MB* that affects the value of the characteristic component of the variability.

Comparison between Macrosporium *and* Botrytis

Both fungi appear to behave in the same way towards all the compounds tested. An exception exists in the case of silver, which gives no significant difference with *Botrytis* as between the ions Ag^+ and $[Ag.2SC(NH_2)_2]^+$. But even here there is no reason to deny the possibility that a small difference of variability may exist, insufficient to be revealed by experiments made on so small a scale. This similarity of behaviour suggests that the preference for thiourea-complexes may be a general phenomenon in the absorption of certain metals by fungal spores.

Comparison of different metals

The arguments which lead us to suggest that copper is readily absorbed by fungal spores in the form of the ion $[Cu2SC(NH_2)_2H_2O]^+$ apply also to zinc, the ions being $[Zn.2SC(NH_2)_2]^{++}$. In the case of silver, however, the same argument suggests that the dithioureargentous ion $[Ag.2SC(NH_2)_2]^+$ requires, at least in the case of *M. sarcinaeforme*, to be broken down to Ag^+ before absorption, and it may be that this must be further built up into some other complex, the nature of which might be elucidated by further research on the same lines. The same applies to the tetrathioureomercuric ion.

The two types of variation considered as limiting cases of a more general relation

In many branches of pharmacology it has been found that toxic or other substances are absorbed by living cells in accordance with the Langmuir adsorption equation (see Clark, 1933), which may be stated in the form

$$\kappa x^n = \frac{r}{1-r}, \tag{1}$$

where κ is a constant, x the concentration applied, n the number of molecules in the free state combined into one fixed molecule, and r the proportion of the total fixing capacity of the material used up, at the given value of x. From this it follows that

$$n \log x = \log \frac{r}{1-r} - \log \kappa \tag{2}$$

$$= 2\left(2r - 1 + \frac{(2r-1)^3}{3} + \frac{(2r-1)^5}{5} + \ldots\right) - \log \kappa,$$

if $2r-1$ is small (between $\pm 0{\cdot}6$), this approximates within 1 % to

$$n \log x \simeq 2(2r-1) - \log \kappa, \tag{3}$$

from which it follows that, κ being constant, $\log x$ will follow the same distribution as r (although with altered moments).

If $n=1$, equation (1) can be rewritten

$$x = \frac{r}{\kappa(1-r)}, \tag{4}$$

which, if r is much less than unity, approximates to

$$x \simeq \frac{r}{\kappa}, \tag{5}$$

from which it follows that x follows the same distribution as r. If, then, the value of r corresponding to the minimum lethal dose for individual spores is normally distributed, a mean $\bar{r}$ and a standard deviation σ_r such that very few individual r values fall outside the range 0·2 to 0·8 will give a statistically homogeneous *logarithmic* distribution, while a very low value for $\bar{r}$ and σ_r will give a *simple normal* distribution of the tolerance. Intermediate cases might occur, but it is doubtful whether experiments of the magnitude of those reported here would suffice to distinguish them.

If Langmuir's equation can in fact be applied in the present case, it would therefore seem that the spores of *Macrosporium* can fix relatively large quantities of silver (which shows direct variation) than of copper, zinc, nickel or mercury (to which variation is logarithmic), while those of *Botrytis* have by the same argument a particular capacity for zinc.

Analysis of the effect of environmental inconstancy

It has already been stated that owing to uncontrolled variations in the temperature of the stock cultures, heterogeneity was introduced into certain of these results, which could be entirely eliminated, in cases where logarithmic variation obtained, by adjustment of the dosage values. This proves that in these cases, of the two degrees of freedom which can contribute towards the heterogeneity of two sets of homogeneous data when combined, that referable to the position of the regression line carries all but an insignificant part of the heterogeneity and therefore the slope of the regression line, b, and hence v, has not been significantly affected.

This can further be demonstrated by calculating the values of b for separate experiments, and demonstrating that the differences are insignificant; this can also be done for b' in cases of direct variation. Numerical data for these calculations are provided in Tables 10 and 12 (in the latter it can be assumed that (as is the case with *Macrosporium*) the effects of mercuric chloride and acetate are the same). It can be shown, not only that b and b' are insignificantly different, but that the values of bm from Table 12 are significantly different, while those of b'/m from Table 10 are nearly significant ($P=0{\cdot}06$).

Thus, in each case, the variability estimated by the appropriate statistic is at any rate less affected by the environmental inconstancy than certain other parameters of dispersion, and definitely less so than the mean. This tends to suggest that the variability represents some characteristic of the spores less likely to be affected by temperature than their mean tolerance; as would be the case if it were determined not wholly by quantitative changes in the behaviour of the spores, but in part by the number of reactions through which the metallic atoms must pass prior to absorption.

Summary

1. Proofs are offered of two theorems relating the chemical reactions of toxic substances to certain statistics of the distribution of the tolerance of fungal spores to them. The term 'variability' is defined, in relation to two special cases.

2. Methods of culturing two species of fungi as test organisms are described, and the experimental details of the fungicidal tests made are given. The preparation of a number of complex salts containing thiourea and various metals is also described.

3. The method of statistical computation of the data is described, together with the relevant tests and criteria of significance. The statistics ultimately calculated were the variance (where the tolerance of the spores to the given metal proved to be normally distributed as its logarithm) and the relative variance (where the tolerance was itself normally distributed). The mean tolerance was also calculated, and certain anomalies in its value in certain cases are noted, and attributed to imperfect control of temperature of the test cultures.

4. From the results, using the theorems of the composition of variability referred to above, it was inferred that:

(*a*) Copper is absorbed by the spores more readily in the form of the monohydrated dithioureocuprous ion, or a related compound, than as the simple cupric ion; certain cupric complex compounds tested appeared to require decomposition before absorption.

(*b*) Zinc is more readily absorbed as the dithioureozinc ion.

(*c*) Silver in the form of the dithioureargentous ion requires decomposition (at least in one case) before absorption.

(*d*) Mercury in the form of the tetrathioureomercuric ion requires decomposition before absorption. All these conclusions are put forward only as the simplest explanation of the facts.

5. A suggestion is put forward relating the observed distribution of tolerance to the Langmuir adsorption equation.

6. Evidence is presented that temperature fluctuations affected the variability (as defined in § II) less than some other statistics, and failed to induce any significant anomalies in it.

I am indebted to Mr E. H. Wilkinson and Mr C. J. Hickman of this Station for the original cultures of *Macrosporium sarcinaeforme* and *Botrytis allii*, and to Dr R. L. Wain for samples of the complex cupric compounds mentioned. I also gratefully acknowledge helpful criticisms and suggestions made by Dr H. Martin, Dr R. L. Wain and Mr H. Todd in the preparation of this paper. The thiourea used in the chemical preparations was kindly supplied by Messrs Hardman and Holden, Ltd. I also have to thank Prof. B. T. P. Barker for permission to work in his laboratory.

REFERENCES

Beilstein, F. (1893). *Handbuch der organischen Chemie*, 3. Aufl. 1318.

Bliss, C. I. (1935*a*). Calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

—— (1935*b*). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

Clark, A. J. (1933). *The Mode of Action of Drugs on Cells.* Arnold.

Evans, A. C. & Martin, H. (1935). The incorporation of direct with protective fungicides. I. The laboratory evaluation of water-soluble wetting agents as constituents of combined washes. *J. Pomol.* **13**, 261.

Horsfall, J. G., Marsh, R. W. & Martin, H. (1937). Studies upon the copper fungicides. IV. The fungicidal value of the copper oxides. *Ann. appl. Biol.* **24**, 867.

McCallan, S. E. A. & Wilcoxon, F. (1930). Studies in fungicides. III. The solvent action of spore excretions on protective copper fungicides. *Mem. Cornell agric. Exp. Sta.* no. 128.

—— —— (1936). The action of fungal spores on Bordeaux mixture. *Contr. Boyce Thompson Inst.* **8**, 151.

Riley, H. L. (1930). Studies on complex salts. III. The effect of alkyl substitution on the tendency of the malonate ion to co-ordinate with copper. *J. chem. Soc.* p. 1649.

RILEY, H. L. & GALLAFENT, V. (1931). Studies on complex salts. IV. The effect of alkyl substitution on the tendency of the aminoacetate ion to co-ordinate with copper. *J. chem. Soc.* p. 2029.

ROSENHEIM, A. & LOEWENSTAMM, W. (1903). Thiocarbamide derivatives of univalent metals and salts. (Abstract in *J. chem. Soc.* **84**, I, 325.)

ROSENHEIM, A. & MEYER, V. J. (1906). Compounds of thiocarbamide with salts of bivalent metals. (Abstract in *J. chem. Soc.* **90**, I, 407.)

ROSENHEIM, A. & STADLER, W. (1906). Compounds of thiocarbamide and xanthamide with salts of univalent copper. (Abstract in *J. chem. Soc.* **90**, I, 407.)

(*Received* 1 *May* 1941)

REVIEWS

Biological Aspects of Infectious Disease. By F. M. BURNETT. Pp. vii + 310. Cambridge: at the University Press. 1940. 15*s*.

Medical science, to its own serious disadvantage, has long tended to hold itself rather aloof from common or garden biology. Realizing this, Dr Burnett, who is a medical immunologist, has set out to consider his own field of interest from the point of view of a biologist rather than of a medical man. Infectious disease whether in plants or animals is essentially a manifestation of the interaction of living things in relation to internal and external factors and, as the author states in his preface: 'It is possible that the biological approach gives a better starting point for the professional study of human infectious disease than a purely medical one.' The result is an extremely interesting book which, however, might have been even better had the author's 'biological approach' been a little wider and deeper than a purely ecological one.

The book is divided into six parts. In the two chapters of Part I are discussed certain general biological considerations such as the ecological point of view with regard to disease, and the evolution of infection and defence. Part II contains three chapters on 'the aggressors'—bacteria, protozoa, and viruses, with worms dismissed in four lines and fungi in three. These two parts, which contain the general ground-work of the subject, make easy and pleasant reading, but they are a little thin and superficial and not infrequently show a tendency to definiteness of viewpoint or statement on questions which are still moot. In Part III, which contains five chapters dealing with 'the processes of defence', the author gives a beautifully clear and simple account of the principles of medical immunology. Part IV deals with 'the natural history of infectious disease' and, to the more general reader, these seven chapters will be, perhaps, the most interesting in the book. Until a comparatively short time ago the scientific study of epidemiology had been much neglected but it is now a rapidly advancing front which, as the author shows, is providing important and even startling results. Part V, which deals with 'some important infectious diseases', illustrates the facts and principles set out in Part IV. Of the seven chapters one each is devoted to a biological consideration of diphtheria, influenza, tuberculosis, plague, cholera, malaria, and yellow fever. Part VI, entitled 'epilogue', is a single chapter in which the author puts forward some rather personal and not always convincing ideas and speculations on 'new diseases and the outlook for the future'.

Dr Burnett admits (p. 95) that 'We are really more ignorant than sometimes we seem', but he writes in so interesting and persuasive a manner and so limpidly does the text flow, that in reading the book one has continually to jerk oneself back into a more severely critical frame of mind. Human infectious disease as seen from the biological standpoint presents interesting analogies with plant disease, and any plant pathologist reading this book will continually find himself making detailed comparisons and contrasts, especially as the author has the knack of condensing a particular situation or disease relationship into a short happy phrase. The manuscript was completed in 1938 although the book was not published until last year: after 21 months of war certain speculations in the 'epilogue' still remain irrelevant or unproven. The book is illustrated by 4 plates and 9 text-figures: no references are given for any statements made in the text.

W. B. BRIERLEY

Agriculture in Uganda. By the Staff of the Department of Agriculture, Uganda. Edited by J. D. TOTHILL. Pp. xvi + 551. Oxford University Press. 1940. 20*s*.

Twenty-five primary authors and thirty-six collaborators have combined in the writing of this remarkable book, the production of which reflects very great credit on the Uganda Department of Agriculture. It is a companion volume to *Uganda* by H. B. Thomas and R. Scott published in 1935, and, together, the two books form a splendid survey of the Protectorate.

The encyclopaedic scope of the volume can best be indicated by outlining the contents. Section I is a brief introduction by the Editor; II, general agriculture—topography and vegetation, climate, native agriculture, soils, soil erosion problems, manures; III, experiment stations and farms; IV–VII, native food crops—bananas, root crops (sweet potatoes, cassava, yams), cereals (bulo, maize, sorghum, bulrush millet, rice, wheat), miscellaneous (ground-nuts, simsim, pulse crops); VIII, cotton; IX,

robusta and other coffees; X, *arabica* coffee; XI, sugar; XII, tobacco; XIII, plantation crops—cacao, rubber, tea; XIV, oil-yielding plants; XV, spices, condiments, drugs; XVI, fibres; XVII, cover-crops and shade trees; XVIII, fruits and vegetables; XIX, grasses and grazing, weeds; XX, bees and locusts; XXI, marketing; XXII, agricultural education and extension work. There is a good synoptic Contents, separate indexes to the scientific names of plants (about 600 entries), and of insects (about 140 entries), and a good general index. The book is illustrated by 5 maps and charts, 9 text-figures, and 30 plates containing reproductions of unusually good photographs.

Each crop is considered in a more or less standardized way: history and general development of the industry, agronomy, breeding and varieties, pests, diseases. Much of the information is taken from reports, bulletins, etc., but it is exceedingly useful to have these reliable data assembled in so convenient a form. On the other hand the book contains much new material, especially on insect pests, and these latter sections will seem to many readers disproportionately lengthy and their detail out of balance.

From every point of view the book is a notable achievement and it will long remain the standard work of reference on agriculture in Uganda. W. B. BRIERLEY

Elements of Botanical Microtechnique. By J. E. Sass. Pp. ix+222. Figs. 33. London: McGraw-Hill Publishing Co., Ltd. 1940. 17*s.* 6*d.*

A well arranged and thoroughly practical training manual which will be found very useful by teachers, advanced or honours students of botany, or by those commencing botanical research. The first half of the book deals clearly and concisely with general principles and methods—collecting and subdividing plant materials for processing; killing, fixing, and storing plant tissues; dehydration for embedding; infiltration and embedding in paraffin; microtome sectioning of material in paraffin; staining paraffin sections; infiltration and embedding in celloidin; sectioning and staining material embedded in celloidin; sectioning unembedded tissues; the preparation of whole mounts; criteria of successful processing. Part II is an interesting and rather unorthodox consideration of specific methods and materials recommended for use in the study of—vegetative organs of vascular plants; Thallophyta; Bryophyta; reproductive structures of Pteridophyta, reproductive structures of Spermatophyta. There are also admirable chapters on the construction, care, and use of the microscope, and on photomicrography. The book closes with a bibliography of forty-one citations and an index.

W. B. BRIERLEY

German-English Science Dictionary for Students in the Agricultural, Biological and Physical Sciences. By L. De Vries. Pp. x+473. London: McGraw-Hill Publishing Co., Ltd. 1939. 18*s.* 0*d.*

French-English Science Dictionary for Students in the Agricultural, Biological and Physical Sciences. By L. De Vries. Pp. viii+546. London: McGraw-Hill Publishing Co., Ltd. 1941. 24*s.* 0*d.*

These useful dictionaries, which rank among the best of their kind, have been compiled by Prof. De Vries of Iowa State College, with the close collaboration of Members of the Graduate Faculty of that Institution. The German-English volume contains 48,000 entries, and the French-English volume 43,000. The works are not, therefore, complete, but they include a very large number of compound terms, and all the basic information needed in finding the meanings of further compounds. Both volumes are built on the same plan—introduction, bibliography of dictionaries, glossaries and reference works, text of dictionary in double column, list of abbreviations with foreign and English equivalents. The text is accurately and clearly printed and the English equivalents adequate and yet not so numerous that a student is bewildered by too many meanings. The books are well produced and of convenient size. W. B. BRIERLEY

Insect Transmission of Plant Diseases. By J. G. Leach. Pp. xviii + 615. London: McGraw-Hill Publishing Co., Ltd. 1940. 42*s.* 0*d.*

It is just half a century ago that Waite advanced the first experimental proof of insect transmission of a plant disease. During the ensuing thirty years numerous sporadic references to the vector function of insects appeared in the rapidly expanding literature of plant diseases and pests but, owing to the disparate training of phytopathologists and applied entomologists, no serious or consistent study was made of the problem. During the last two decades, however, largely owing to the attention focused on virus diseases, the question of insect vectors increasingly aroused attention and our knowledge of this subject was greatly enlarged. The time was ripe for a survey and co-ordination of the widely scattered data and viewpoints and in this book Prof. Leach, who is one of the leading investigators in this field, has carried out this difficult task in a very commendable way. Fortunately, the author visualized his task in no myopic perspective and, although his theme is specifically the transmission of plant diseases by insects, he has approached the subject from a broad biological standpoint and treated it in a comprehensive and philosophical manner.

Following an introductory chapter there are four rather general chapters dealing respectively with the wider interrelationships of plants and insects, symbiosis between insects and micro-organisms and its significance in plant pathology, the relation of insects to the spread and development of plant diseases, and plant diseases caused by toxicogenic insects. Much of Ch. 2 seems perhaps a little unnecessary but it is very pleasant to see again so many of the old Kerner and Oliver illustrations. The next five chapters discuss the vector function of insects in numerous specific diseases caused by bacteria, fungi, viruses, and protozoa, whilst Ch. 11 deals with mites, nematodes, and other small animals as vectors of plant diseases. English readers may, perhaps, find it a little surprising that a page and a half of the latter chapter is devoted to birds as vectors of mistletoe but, of course, American works on plant disease are usually more catholic than their English compeers. The next four chapters cover a wide yet detailed and very important field in a kind of Box and Cox arrangement—the anatomy and physiology of plants in relation to infection and insect vectors, the anatomy and physiology of insects in relation to the transmission of plant diseases, the inocula of plant pathogens in relation to insect transmission, and the feeding and breeding habits of insects in relation to the transmission of plant diseases. All four chapters seem to me a little thin. There is then a somewhat naïve but mildly interesting chapter comparing the insect transmission of diseases in animals and in plants, and the final chapter is an outline of methods useful in the study of insect transmission of plant diseases. In a 21-page appendix numerous facts of insect transmission of plant diseases are tabulated in a form convenient for reference. There is a glossary of 225 terms and an index. Each chapter terminates with a list of references of which about three quarters are U.S.A. publications, and in which a little more attention should be given to standardization of contracted titles. The book is illustrated by a frontispiece and 238 text-figs., many of which are original. The text and especially the legends to illustrations contain an irritating number of misprints.

Most of the text is just good, clear, descriptive writing and the book marshalls and co-ordinates a great number of facts, ideas, and viewpoints which are critically reviewed and evaluated. After reading the book the general picture left in my mind tends to support the author's view that 'as a biological phenomenon the association of insects and plant pathogens is in many respects similar to that of entomophily'. Certainly, the insect vector relationship is not only more practically important but it is much more fundamental than I and, I rather think, most of us who are interested in plant disease had realized.

W. B. BRIERLEY

LIST OF MEMBERS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

(Changes of address should be notified immediately to the General Secretary and the Treasurer)

HONORARY MEMBERS

APPEL, Geh. Reg.-Rat Prof. Dr OTTO, Biologische Reichsanstalt f. Land- u. Forstwirtschaft, Dahlem, Berlin.
CHEVALIER, Directeur Aug., Laboratoire d'Agronomie Coloniale, Rue Cuvier 57, Paris (V^e^).
ESCHERICH, Prof. Dr KARL, Institut für Angewandte Zoologie, München, N.W. 2, Germany.
GÄUMANN, Prof. ERNST, Eidgenösschen Technischen Hochschule, Universitätsstrasse, Zurich, Switzerland.
HOPKINS, Dr A. D., Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.
HOWARD, Dr L. O., late Principal Entomologist, Department of Agriculture, Washington, D.C., U.S.A.
JONES, Prof. L. R., University of Wisconsin, Madison, U.S.A.
MARCHAL, Prof. P., Station Entomologique, Paris, France.
NILSSON-EHLE, Prof. N. H., University of Lund, Svalöv, Sweden.
SILVESTRI, Prof. F., R. Scuola Sup. d'Agricoltura di Portici, Naples, Italy.
UVAROV, B. P., D.Sc., Imperial Institute of Entomology, British Museum (Natural History), Cromwell Road, South Kensington, S.W. 7.
VAVILOV, Dr N. I., Director, Institute of Plant Industry, Rue Herzen 44, Leningrad, U.S.S.R.

ORDINARY MEMBERS (*Life Members are marked* *)

1931 AINSWORTH, G. C., B.Sc., Ph.D., Imperial Mycological Institute, Kew, Surrey. (Council 1940– .)
1920 ALCOCK, Mrs N. L., M.B.E., F.L.S., 61 Holywell, Oxford.
1936 ALLEN, G. STAFFORD, Stafford Works, Long Melford, Suffolk.
1928 ALLEN, L. A., M.Sc., Ph.D., Water Pollution Research Laboratory, Langley Road, Watford, Herts.
1939 ASHWORTH, Miss DOROTHY, M.Sc., Ph.D., R.H.S. Laboratories, Wisley, Ripley, Surrey.
1935 BAILEY, K. F. GOODWIN, D.I.C., Bayer Products Ltd., Africa House, Kingsway, London, W.C. 2.
1926 BALFOUR-BROWNE, Prof. F., M.A., F.R.S.E., Brae, Dumfries, Scotland.
1914 BARKER, Prof. B. T. P., M.A., Director, Research Station, Long Ashton, Bristol. (Council 1916–20, 1926–8.)
1931 BARNARD, Mrs DOROTHEA E., c/o B.O.C. (Burma Concession Ltd.), Khodaung, Upper Burma.
1927 BARNES, H. F., M.A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts. (Zoological Secretary 1939– ; Council 1936–8.)
1922 BARRATT, Miss K., D.Sc., Principal, Horticultural College, Swanley, Kent (temporarily at Midland Agricultural College, Sutton Bonington).
1938 BATES, G. H., D.Sc., The Farm Institute, Penkridge, Stafford.
1930 BAWDEN, F. C., M.A., Rothamsted Experimental Station, Harpenden, Herts.
1932 BEAUMONT, A., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
1928 BENNETT, F. T., D.Sc., Ph.D., N.D.A., Agricultural Department, King's College, Newcastle-upon-Tyne, 2.
1941 BENNETT, S. H., B.Sc., Long Ashton Research Station Field Laboratory, Abbey Road, Evesham.
1919 BEWLEY, W. F., C.B.E., V.M.H., D.Sc., Director, Experimental and Research Station, Cheshunt, Herts. (Council 1922–4, 1929.)
1932 BLACKMAN, G. E., M.A., Botany Department, Imperial College of Science, London, S.W. 7. (Vice-President 1940; Council 1939– .)
1919 BLACKMAN, Prof. V. H., M.A., Sc.D., F.R.S., 17 Berkeley Place, Wimbledon, S.W. 18. (President 1924–5; Vice-President 1922–3; Council 1920–1, 1926–31.)

1935 Bodenheimer, Prof. F. S., D.Phil., Department of Zoology, Hebrew University, Jerusalem, Palestine.
1940 Bond, G., B.Sc., Ph.D., Department of Botany, The University, Glasgow, W. 2.
1939 Bond, T. E. T., M.Sc., Ph.D., Tea Research Institute of Ceylon, St Coombs, Talawakelle, Ceylon.
1935 Booer, J. R., F. W. Berk & Co. Ltd., Commonwealth House, 1–19 New Oxford Street, London, W.C. 1.
1937 Bovingdon, H. H. S., Ph.D., D.I.C., A.R.C.S., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks.
1937 Braybrook, F. H., B.A., Technical Products Ltd., 29 Great St Helen's, London, E.C. 3.
1919 Brenchley, Miss W. E., D.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts. (Vice-President 1941; Council 1940– .)
1939 Brett, C. C., M.A., The Official Seed Testing Station, Huntingdon Road, Cambridge.
1914 Brierley, Prof. W. B., D.Sc., F.L.S., Department of Agricultural Botany, The University, Reading. (President 1932–3; Vice-President 1930–1; General and Botanical Secretary 1919–22; General and Botanical Editor 1921– .)
1914 Brooks, Prof. F. T., M.A., LL.D., F.R.S., F.L.S., The Botany School, Cambridge. (Vice-President 1928–9; Council 1921–2, 1927–30.)
1921 Brooks, R. St-John, M.D., M.A., D.P.H., D.T.M. and H., Lister Institute, Chelsea Bridge Road, London, S.W. 1.
1933 Brown, J. M. B., B.Sc., Imperial Forestry Institute, Oxford.
1938 Brown, Miss M. R., Ph.D., Newnham College, Cambridge.
1924 Brown, Prof. W., M.A., D.Sc., F.R.S., Imperial College of Science, London, S.W. 7. (Vice-President 1933, 1935; Botanical Secretary 1928–32; Council 1933–5.)
1920 Buddin, W., M.A., Agricultural Advisory Offices, The University, Reading. (Council 1932–4.)
1938 Bumpus, E. D., Ph.D., Heatherdene, Moorlands Road, West Moors, Wimborne, Dorset.
1928 Burr, S., M.Sc., Department of Agriculture, The University, Leeds.
1935 Busvine, J. R., B.Sc., A.R.C.S., The Bureau of Entomology, 41 Queen's Gate, London, S.W. 7.
1940 Butler, C. G., M.A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1920 Butler, Sir Edwin, C.M.G., C.I.E., D.Sc., LL.D., M.B., F.R.S., F.L.S., Woodlands, Clapham, Bedford. (President 1928–9; Vice-President 1924–5; Council 1921–5, 1930–1.)
1930 Caldwell, J., D.Sc., Ph.D., Department of Botany, University College, Exeter.
1934 Cameron, A. E., M.A., D.Sc., Department of Agricultural and Forest Zoology, The University, Edinburgh, 8.
1932 Campbell, A. H., Ph.D., The University, Bristol.
1928 Cann, F. R., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks (temporarily at Pest Infestation Laboratory, Biological Field Station, Slough).
1937 Capoor, S. P., M.Sc., Ph.D., Department of Plant Pathology, College of Agriculture, Poona 5, India.
1929 Cartwright, K. T. St George, M.A., D.I.C., F.L.S., Forest Products Research Laboratory, Princes Risborough, Bucks. (Council 1931–3.)
1905 Chandler, S. E., D.Sc., F.L.S., 59 Anerley Park, Penge, S.E. 20. (Secretary 1911–13; Council 1914–20.)
1925 Cheal, W. F., D.I.C., N.D.A., Gosmoor Lane, Elm, Nr. Wisbech, Cambs.
1926 Cheesman, Prof. E. E., M.Sc., A.R.C.S., Imperial College of Tropical Agriculture, Trinidad, B.W.I.
1930 Chesters, C. G. C., M.Sc., Ph.D., Department of Botany, The University, Birmingham.
1908 Chittenden, F. J., V.M.H., F.L.S., Royal Horticultural Society, Vincent Square, London, S.W. 1. (Council 1914–21.)
1921 Chrystal, R. N., M.A., D.Sc., Imperial Forestry Institute, Oxford. (Council 1931–3.)
1936 Cohen, M., M.Sc., Ph.D., Department of Agricultural Entomology, The University, Manchester 13.
1939 Comrie, L. C., B.Sc., Toklai Experimental Station, Cinnemara P.O., Assam, India.
1937 Cone, Mrs G. B., M.Sc., 1 Waipapa Terrace, Wellington, E. 2, New Zealand.
1905 Cornwallis*, F. S. W., D.L., J.P., Linton Park, Maidstone, Kent.
1935 Crauford-Benson, H. J., B.Sc., D.I.C., The Cooper Field Research Station, Little Gaddesden, Berkhamsted, Herts.

1941 Croxall, H. E., B.Sc., Research Station, Long Ashton, Bristol.
1920 Cunliffe, N., M.A., D.Sc., School of Rural Economy, The University, Oxford. (Council 1927–9.)
1929 Curtis, Miss K. M., M.A., D.Sc., D.I.C., F.L.S., Cawthron Institute, Nelson, New Zealand.
1927 Dade, H. A., A.R.C.S., Imperial Mycological Institute, Kew, Surrey. (Council 1938–9.)
1939 David, W. A. L., B.A., London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London.
1941 Davies, D. Walters, B.Sc., Agricultural Buildings, University College of Wales, Aberystwyth.
1930 Dawson, R. B., M.Sc., F.L.S., Director, St Ives Research Station, Bingley, Yorks.
1936 Dennis, R. W. G., B.Sc., Ph.D., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh 12.
1923 Dixon*, Miss A., M.Sc., Kanguri, Batchwood Drive, St Albans, Herts.
1920 Dowson, W. J., M.A., D.Sc., The Botany School, Cambridge. (Council 1937–9.)
1937 Dowson, Mrs W. J., 26 Barrow Road, Cambridge.
1935 Duffield, C. A. W., F.R.E.S., Pickersdene, Brook, Ashford, Kent.
1940 Dyke, F. M., B.Sc., Lever Bros. and Unilever Ltd., Unilever House, Blackfriars, London, E.C. 4.
1927 Edwards, E. E., D.Sc., Department of Agricultural Zoology, University College, Cardiff.
1935 Ekins, Miss E. H., B.Sc., Principal, The College, Studley, Warwickshire.
1934 Engledow, Prof. F. L., C.M.G., M.A., School of Agriculture, Cambridge.
1939 Erith, Miss A. G., B.Sc., Ph.D., Department of Agricultural Botany, The University, Reading.
1935 Evans, A. C., B.Sc., Ph.D., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1920 Fahmy, T., D.Sc., Director, Plant Pathology Section, El Dokki, Cairo, Egypt.
1920 Fenton, E. Wyllie, M.A., D.Sc., Department of Botany, Edinburgh and East of Scotland College of Agriculture, 13 George Square, Edinburgh.
1929 Findlay, W. P. K., D.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks. (Botanical Secretary 1936; General and Botanical Secretary 1937– .)
1935 Fish, S., M.Agr.Sc., Department of Agriculture, Biological Branch, Plant Research Laboratory, Burnley Gardens, Swan Street, Burnley, E. 1, Victoria, Australia.
1919 Fisher, K., The School, Oundle, Northampton.
1923 Fisher, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks. (Vice-President 1936; Council 1934–6.)
1931 Foister, C. E., B.A., Ph.D., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh 12.
1920 Fox-Wilson, G., N.D.Hort., F.L.S., R.H.S. Laboratories, Wisley, Ripley, Surrey. (General and Zoological Secretary 1935–6; Zoological Secretary 1933–4, 1937–8; Council 1927–9.)
1936 Franklin, Miss M. T., B.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans, Herts.
1940 Freeman, J. A., Ph.D., Imperial College Biological Field Station, Slough, Bucks.
1913 Fryer, J. C. F., O.B.E., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (President 1926–7; Treasurer 1914–19; Council 1921, 1924–5.)
1933 Gadd, C. H., D.Sc., Tea Research Institute of Ceylon, St Coombs, Talawakelle, Ceylon.
1930 Galloway, L. D., M.A., c/o Midland Bank, Wimbledon Hill, S.W. 19.
1935 Garrett, S. D., M.A., Rothamsted Experimental Station, Harpenden, Herts. (Council 1939–40.)
1927 Gibson, W. H., O.B.E., D.Sc., F.I.C., F.Inst.P., Research Department, Royal Arsenal, Woolwich.
1938 Gill, N. T., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.
1937 Gillespie, J., B.Sc., County School, Cardigan, Wales.
1937 Gillespy, T. G., B.Sc., A.R.C.S., University of Bristol Fruit and Vegetable Preservation Research Station, Campden, Glos.
1920 Gimingham, C. T., O.B.E., B.Sc., F.I.C., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (President 1938–40; Vice-President 1937; Zoological Editor 1934– ; Council 1929–32.)
1941 Glasscock, H. H., M.Sc., Dip. Agric. (Wye), South-Eastern Agricultural College, Wye, Kent.
1920 Glynne, Miss M. D., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1938 Goodall, D. W., B.Sc., D.I.C., East Malling Research Station, East Malling, Kent.
1920 Goodey, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans, Herts. (President 1934–5; Council 1923–9, 1936–8.)

1939 Goodliffe, E. R., B.A., 25 Prospect Road, New Barnet, Herts.
1939 Gough, H. C., Ph.D., Department of Agriculture, The University, Leeds.
1929 Grainger, J., Ph.D., Tolson Memorial Museum, Ravensknowle, Huddersfield.
1921 Gray, Prof. P. H. H., M.A., D.Sc., Macdonald College, Quebec, Canada.
1929 Green, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey. (Council 1941– .)
1939 Greenhill, A. W., Ph.D., Boron Agricultural Bureau, 3*a* Harlesden Road, St Albans, Herts.
1940 Greenslade, R. M., B.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent.
1933 Gregor, J. W., Ph.D., F.L.S., Scottish Plant Breeding Station, Craigs House, Corstorphine, Edinburgh.
1939 Gregory, Prof. F. G., D.Sc., F.R.S., Imperial College of Science, London, S.W. 7.
1940 Gregory, P. H., B.Sc., Ph.D., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1934 Grieve, B. J., M.Sc., Ph.D., D.I.C., Botany School, The University of Melbourne, Melbourne, N. 3, Victoria, Australia.
1934 Güssow, H. T., LL.D., F.R.S.C., F.L.S., Dominion Botanist, Department of Agriculture, Central Experimental Farm, Ottawa, Canada.
1920 Gwynne-Vaughan, Prof. Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.
1930 Hall, W. J., D.Sc., A.R.C.S., Director, Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.
1924 Harris, R. V., B.Sc., A.R.C.S., D.I.C., East Malling Research Station, East Malling, Kent. (Council 1940– .)
1938 Harvey, G. L., B.Sc., A.R.C.S., Asiatic Petroleum Co. Ltd., Great St Helens, London, E.C. 3.
1927 Hatton, R. G., C.B.E., V.M.H., M.A., D.Sc., East Malling Research Station, East Malling, Kent. (Council 1928.)
1940 Hawker, Miss L. E., Ph.D., Imperial College Biological Field Station, Slough, Bucks.
1938 Henderson, F. Y., D.Sc., Imperial College of Science, London, S.W. 7.
1941 Herford, G. V. B., B.A., M.Sc., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.
1933 Hey, G. L., M.A., The Murphy Chemical Co., Wheathampstead, Herts.
1940 Hickman, C. J., M.Sc., Long Ashton Research Station Field Laboratory, Abbey Road, Evesham.
1940 Higgins, A. E. H., A.R.C.S., D.I.C., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.
1920 Hill, Sir Arthur, K.C.M.G., M.A., Sc.D., D.Sc., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.
1920 Hiscox, Miss E. R., M.Sc., National Institute for Research in Dairying, Shinfield, Reading.
1935 Hodson, W. E. H., A.R.C.S., D.I.C., Agricultural Advisory Offices, The University, Reading. (Council 1941– .)
1937 Holborn, J. M., B.Sc., A.R.C.S., Jeyes' Sanitary Compounds Co. Ltd., Richmond Street, Plaistow, London, E. 13.
1938 Holman, H. J., B.Sc., A.R.C.S., A.I.C., Imperial Institute, South Kensington, London, S.W. 7.
1920 Horton, E., B.Sc., F.I.C., 1 Sispara Gardens, West Hill Road, Wandsworth, London, S.W. 18.
1941 Howe, R. W., B.Sc., A.R.C.S., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.
1927 Howes, F. N., D.Sc., Royal Botanic Gardens, Kew, Surrey. (Council 1930–1.)
1928 Hughes, A. W. McKenny, D.I.C., Natural History Museum, South Kensington, London, S.W. 7.
1936 Hull, R., B.Sc., Ph.D., A.R.C.S., Midland Agricultural College, Sutton Bonington, Loughborough.
1938 Hunter-Smith, J., M.B.E., B.Sc., N.D.A., Principal, Herts Institute of Agriculture, Oaklands, St Albans, Herts.
1940 Hurst, H., B.Sc., Imperial College Biological Field Station, Slough, Bucks.
1941 Jacob, F. H., M.Sc., University College of North Wales, Memorial Buildings, Bangor.
1927 Jacobs, S. E., Ph.D., Bacteriology Department, Imperial College of Science, London, S.W. 7.
1927 Jary, S. G., B.A., South-Eastern Agricultural College, Wye, Kent. (Council 1934–6.)
1934 Johnson, C. G., B.Sc., 2 Aldcliffe Place, Lancaster.
1938 Johnson, L. R., M.Sc., Department of Agriculture, The University, Leeds 2.
1931 Jones, A. Powell, M.Sc., Department of Agriculture, The University, Leeds 2.

1940 JONES, D. PRICE, Ph.D., School of Agriculture, Cambridge.
1934 JONES, E. PARRY, B.Sc., Ph.D., A.I.C.T.A., Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.
1940 JONES, F. G. W., B.A., School of Agriculture, Cambridge.
1927 JONES, G. HOWARD, M.A., Kasenga, Stanley Avenue, Chesham, Bucks.
1927 JOSEPH, E. G., B.Sc., Redcot, Swanley, Kent.
1935 KEARNS, H. G. H., B.Sc., Ph.D., Research Station, Long Ashton, Bristol. (Council 1939– .)
1940 KEAY, Miss M. A., M.A., Ph.D., Department of Agricultural Botany, The University, Reading.
1932 KERR, H. W., M.Sc., Ph.D., Director, Bureau of Sugar Experiment Stations, Brisbane, Australia.
1940 KEYWORTH, W. G., B.Sc., Ph.D., D.I.C., A.R.C.S., East Malling Research Station, East Malling, Kent.
1907 KING, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6 Blythewood Square, Glasgow.
1926 KINGSTON, H. I., Athlone, High Road, Broxbourne, Herts.
1933 KRAMER, L. M. J., M.A., Ph.D., City of London School, Victoria Embankment, London, E.C. 4 (temporarily at Marlborough College, Wilts).
1921 LACEY, Miss M. S., D.Sc., Botany Department, Imperial College of Science, South Kensington, London, S.W. 7.
1935 LADELL, W. R. S., F.I.C., Amity Hall, Alley P.O., Jamaica, B.W.I.
1933 LEACH, R., B.A., Banana Leaf Spot Control Board, Department of Science and Agriculture, Jamaica, B.W.I.
1934 LEAN, O. B., B.Sc., D.I.C., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks. (Council 1940– .)
1926 LE PELLEY, R. H., B.Sc., Ph.D., D.I.C., A.R.C.S., Department of Agriculture, Nairobi, Kenya, Africa.
1920 LLOYD, L., D.Sc., Zoological Department, The University, Leeds 2.
1937 LOVIBOND, Miss C. B. J., B.Sc., Boron Agricultural Bureau, 3*a* Harlesden Road, St Albans, Herts.
1931 MCCLEAN, A. P. D., D.Sc., Natal Herbarium, Berea, Durban, Natal, Union of S. Africa.
1936 MACDONALD, J. A., B.Sc., Ph.D., F.R.S.E., Botany Department, The University, St Andrews.
Orig. MACDOUGALL*, Prof. R. S., M.A., D.Sc., Ivy Lodge, Gullane, E. Lothian, Scotland. (Vice-President 1914–19; Council 1908–13.)
1929 MACGILL, Miss E. I., D.Sc., Linkfield, Hawthorn Road, Denton, Manchester.
1925 MACLEOD, D. J., M.A., Drawer 940, Fredericton, New Brunswick, Canada.
1938 MACLEOD, J., D.Sc., The Cooper Field Research Station, Little Gaddesden, Berkhamsted, Herts.
1920 MANGHAM, Prof. S., M.A., University College, Southampton.
1917 MANN, H. H., D.Sc., F.I.C., F.L.S., Woburn Experimental Station, Husborne Crawley, Bletchley.
1938 MARSH, R. W., M.A., Research Station, Long Ashton, Bristol.
1934 MARSHALL, J., B.Sc., A.R.C.S., A.I.C.T.A., Cotton Experiment Station, P.O. Box 95, Barberton, Transvaal, S. Africa.
1930 MARTIN, H., D.Sc., A.R.C.S., F.I.C., Research Station, Long Ashton, Bristol. (President 1941– ; Vice-President 1938; Council 1936–8.)
1940 MARTIN, J. T., B.Sc., Ph.D., A.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1922 MASON, E. W., M.A., M.Sc., F.L.S., Imperial Mycological Institute, Kew, Surrey.
1927 MASSEE, A. M., D.Sc., East Malling Research Station, East Malling, Kent. (Council 1932–4.)
1939 MASSEY, R. E., F.L.S., Wellswood, Park View Road, Pinner Hill, Middlesex.
1938 MEGGITT, F. J., M.Sc., Ph.D., Department of Zoology and Botany, University College, Rangoon.
1933 MEIKLEJOHN, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1941 METCALFE, C. R., M.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
1923 MILES, H. W., D.Sc., Ph.D., Department of Agricultural Zoology, The University, Manchester 13. (Vice-President 1939; Council 1937–9.)
1921 MILLARD, W. A., D.Sc., Department of Agriculture, The University, Leeds 2.
1941 MILNE, A., M.A., Ph.D., Department of Agriculture, King's College, Newcastle-upon-Tyne 2.
1939 MILNE, P. S., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1940 MONTGOMERY, H. B. S., B.A., Ph.D., D.I.C., East Malling Research Station, East Malling, Kent.

1932 MOORE, Miss E. S., B.Sc., Ph.D., Tobacco Research Laboratory, Balfour, C.P., South Africa.
1940 MOORE, M. H., Dip. Hort. (Reading), East Malling Research Station, East Malling, Kent.
1928 MOORE, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (Treasurer 1940– ; Council 1938–9.)
1941 MORISON, G. D., B.Sc., Ph.D., Advisory Entomological Department, Marischal College, Aberdeen.
1922 MORLAND, D. M. T., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
1920 MORRIS, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.
1920 MOSLEY, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge, Middlesex.
1914 MUNRO, Prof. J. W., M.A., D.Sc., Imperial College Biological Field Station, Slough, Bucks. (Vice-President 1933; General and Zoological Secretary 1928–32; Zoological Editor 1933; Council 1922–4, 1927.)
1938 MUSGRAVE, A. J., B.Sc., D.I.C., Imperial College Biological Field Station, Slough, Bucks.
1925 MUSKETT, Prof. A. E., D.Sc., A.R.C.S., Plant Disease Division, Queen's University, Belfast, Northern Ireland.
1937 NATTRASS, R. M., B.Sc., Ph.D., D.I.C., Department of Agriculture, Nairobi, Kenya.
1928 NEWTON, H. C. F., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop. (Council 1938–40.)
1923 OGILVIE, L., M.A., M.Sc., Research Station, Long Ashton, Bristol.
1925 OLDHAM, J. N., B.Sc., Ph.D., Research Institute in Animal Pathology, Royal Veterinary College, The Beeches, Streatley, Berks. (Council 1934.)
1940 ORDISH, F. G., Dip. Hort., Imperial Chemical Industries, Ltd., Yalding, Kent.
1937 OYLER, Miss E., B.Sc., Experimental and Research Station, Cheshunt, Herts.
1941 PARKER-RHODES, A. F. P., B.A., Agric. Dip. (Camb.), Research Station, Long Ashton, Bristol.
1928 PARKIN, E. A., M.Sc., Ph.D., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks (temporarily at Pest Infestation Laboratory, Biological Field Station, Slough). (Council 1941– .)
1935 PEARL, R. T., B.Sc., A.R.C.S., South-Eastern Agricultural College, Wye, Kent.
1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge. (Vice-President 1940–1; Council 1939– .)
Orig. PETHYBRIDGE, G. H., M.A., B.Sc., Ph.D., Penlee, Harleigh Road, Bodmin, Cornwall. (Vice-President 1926–7; Council 1912–13, 1925–7.)
1915 PORTER*, Miss A., D.Sc., Zoological Society of London, Regent's Park, N.W. 8.
1934 POTTER, C., B.Sc., Ph.D., D.I.C., Rothamsted Experimental Station, Harpenden, Herts. (Council 1941– .)
1907 POULTON*, Prof. Sir EDWARD, M.A., D.Sc., LL.D., F.R.S., Wykeham House, Banbury Road, Oxford. (President 1922–3; Vice-President 1909–13.)
1938 POWELL, A. K., M.Sc., Imperial College Biological Field Station, Slough, Bucks.
1919 PRAIN*, Sir DAVID, Lt-Col., C.M.G., C.I.E., V.M.H., M.A., M.B., LL.D., F.R.S., The Well Farm, Warlingham, Surrey. (President 1919–21.)
1923 PRESTON, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.
1928 RAMSBOTTOM, J., O.B.E., M.A., Dr.Sc., F.L.S., Keeper of Botany, British Museum (Natural History), Cromwell Road, London, S.W. 7.
1931 REICHERT, I., Ph.D., Chief Plant Pathologist, Agricultural Research Station, Rehovet, Palestine.
1941 REYNOLDSON, T. B., B.Sc., Ph.D., Sewage Disposal Works, Deighton, Huddersfield.
1940 RICHARDS, T., Ph.D., Department of Agricultural Bacteriology, The University, Reading.
1941 RITCHIE, Miss W. M., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1921 ROACH, W. A., D.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent. (Council 1935–6.)
1914 ROBERTS, A. W. RYMER, M.A., Applegarth, Cartmel, Grange-over-Sands, Lancs.
1940 ROBERTS, Miss F. M., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1923 ROBINSON, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.
1918 ROBSON, R., Institute of Agriculture, Chelmsford.
1920 ROEBUCK, A., N.D.A., Midland Agricultural College, Sutton Bonington, Loughborough. (Council 1938–40.)
1919 RUSSELL, Sir JOHN, O.B.E., D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts. (Vice-President 1923; Council 1921–3.)

1932 Russell, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.
1939 Sadasivan, T. S., M.Sc., Gokulam, Mylapore, Madras City, India.
1929 Salaman, R. N., M.D., F.R.S., Homestall, Barley, Nr. Royston, Herts.
1935 Sampson, Miss K., M.Sc., Agricultural Buildings, University College of Wales, Aberystwyth.
1923 Samuel, G., M.Sc., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (Council 1935–7.)
1933 Schimmer, Miss F. C., M.Sc., A.R.C.S., D.I.C., Horticultural College, Swanley, Kent (temporarily at Midland Agricultural College, Sutton Bonington, Loughborough).
1941 Selman, I. W., B.Sc., Ph.D., Experimental and Research Station, Cheshunt, Herts.
1938 Shaw, H., M.Sc., Ph.D., East Malling Research Station, East Malling, Kent.
1932 Sheffield, Miss F. M. L., D.Sc., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
1939 Singh, Prof. B. N., D.Sc., Institute of Agricultural Research, P.O. Hindu University, Benares, India.
1928 Small, T., M.Sc., Ph.D., A.R.C.S., Botany Department, The University, Manchester 13.
1940 Smith, A., M.A., B.Sc., Ph.D., Plant Pathological Laboratory, Milton Road, Harpenden, Herts.
1919 Smith, J. Henderson, M.B., Ch.B., B.A., Hill Harden, Harpenden, Herts. (President 1936–7; Treasurer 1931–9.)
1920 Smith, K. M., D.Sc., Ph.D., F.R.S., Director, Plant Virus Research Station, School of Agriculture, Cambridge.
1927 Smith, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahamstown, S. Africa.
1913 South, F. W., M.A., 73 The Fairway, Aldwick Bay, Bognor Regis, Sussex.
1919 Speyer, E. R., M.A., Experimental and Research Station, Cheshunt, Herts. (Council 1930–2, 1937–9.)
1920 Spinks, G. T., M.A., Research Station, Long Ashton, Bristol.
1920 Stapledon, Prof. Sir George, C.B.E., M.A., F.R.S., University College of Wales, Aberystwyth.
1933 Steer, W., B.A., East Malling Research Station, East Malling, Kent.
1936 Steven, W. F., M.A., 8 Dynevor Road, Richmond, Surrey.
1922 Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough.
1919 Stone, H., Messrs Benton and Stone, Bracebridge Street, Birmingham.
1926 Storey, H. H., M.A., Ph.D., East African Agricultural Research Station, Amani, Tanga, Tanganyika Territory, E. Africa.
1940 Storey, I. F., B.Sc., Ph.D., Agricultural Advisory Offices, The University, Reading.
1927 Stoughton, Prof. R. H., D.Sc., A.R.C.S., The University, Reading. (General and Botanical Secretary 1933–4; Botanical Secretary 1935.)
1933 Sutton, M. A. F., F.L.S., Sutton and Sons, Ltd., Reading.
1921 Tattersfield, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1914 Taylor, H. V., O.B.E., V.M.H., D.Sc., Commissioner for Horticulture, Ministry of Agriculture and Fisheries, 23–25 Soho Square, London, S.W. 1. (Council 1927–8.)
1939 Taylor, R. E., B.Sc., Ph.D., The Botany School, Cambridge.
1936 Templeman, W. G., M.Sc., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks.
1927 Thaysen, A. C., Ph.D., Microbiological Section, Chemical Research Laboratory, Teddington. (Council 1933–5.)
1931 Thomas, I., M.Sc., Ph.D., School of Agriculture, University College of North Wales, Memorial Buildings, Bangor.
1938 Thompson, H. W., M.Sc., Department of Agriculture, The University, Leeds 2.
1928 Thompson, W. R., D.Sc., F.R.S., Entomological Laboratory, Belleville, Ontario, Canada. (Vice-President 1930–2; Council 1940.)
1933 Thomson, J. R., B.Sc., School of Agriculture, Khartoum North, Sudan.
1919 Thornton, H. G., B.A., D.Sc., F.R.S., Rothamsted Experimental Station, Harpenden, Herts. (Council 1936–8.)
1933 Thorold, C. A., B.Sc., Department of Agriculture, Port of Spain, Trinidad, B.W.I.
1927 Tincker, M. A. H., M.A., D.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey. (Council 1930–2.)
1938 Turner, Miss E. M. C., B.A., Water Pollution Research Laboratory, Langley Road, Watford, Herts.
1929 Vyvyan, M. C., M.A., East Malling Research Station, East Malling, Kent.

1941 WAIN, R. L., M.Sc., Ph.D., A.I.C., Research Station, Long Ashton, Bristol.
1929 WAKELY, C. T. N., B.Sc., Bayer Products Ltd., Africa House, Kingsway, London, W.C. 2.
1932 WALDIE, J. S. L., B.Sc., N.D.A., Department of Agricultural Botany, The University, Reading.
1923 WALKDEN, H., The Raft, Derbyshire Road, Sale, Cheshire.
1940 WALKER, A. G., B.Sc., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1941 WALKER, E., M.Sc., M.A., D.Phil., A.I.C., Department of Biochemistry, University Museum, Oxford.
1931 WALLACE, E. R., B.A., The Agricultural Institute, Kirton, Nr. Boston, Lincs.
1919 WALLACE, J. C., M.C., Director, The Agricultural Institute, Kirton, Nr. Boston, Lincs.
Orig. WARBURTON, C., M.A., Yew Garth, Grantchester, Cambridge. (Council 1905–13.)
1919 WARE, W. M., D.Sc., South-Eastern Agricultural College, Wye, Kent. (Council 1930–2, 1940– .)
1922 WARINGTON, Miss K., D.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1936 WARNE, Mrs M. M., M.A., 25 Amherst Road, Withington, Manchester.
1930 WATSON, Mrs M. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1918 WEST, C., M.A., D.Sc., A.R.C.S., D.I.C., F.L.S., Ditton Laboratory, East Malling, Kent. (Council 1931.)
1940 WESTERN, J. H., B.Sc., Ph.D., Botany Department, The University, Manchester 13.
1923 WESTON, W. A. R. DILLON, M.A., Ph.D., School of Agriculture, Cambridge.
1937 WHITE, H. L., M.A., Ph.D., D.I.C., Experimental and Research Station, Cheshunt, Herts.
1921 WHITEHEAD, T., M.Sc., Ph.D., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
1937 WICKENS, G. M., B.Sc., Ph.D., Tobacco Research Station, P.B. 74B, Salisbury, S. Rhodesia.
1937 WILKINS, W. H., M.A., D.Phil., F.L.S., Department of Botany, The University, Oxford.
1941 WILKINSON, E. H., B.Sc., Research Station, Long Ashton, Bristol.
1912 WILLIAMS, C. B., M.A., Sc.D., Rothamsted Experimental Station, Harpenden, Herts. (Vice-President 1934–5; Council 1933–5.)
1930 WILLIAMS, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (Council 1934–6.)
1941 WILLIAMSON, K. B., M.A., 7 Attimore Road, Welwyn Garden City, Herts.
1923 WILSON, Miss A. P., M.B.E., A.R.C.S., 7*a* Arlington Drive, Nottingham.
1933 WILSON, A. R., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.
1914 WILSON, M., D.Sc., A.R.C.S., F.R.S.E., F.L.S., Royal Botanic Gardens, Edinburgh.
1921 WILTSHIRE, S. P., M.A., D.Sc., Director, Imperial Mycological Institute, Kew, Surrey. (Vice-President 1936–7; Council 1926–9, 1935–7.)
1932 WOOD, J., A.R.C.S., D.I.C., The Agricultural Institute, Kirton, Nr. Boston, Lincs.
1939 WOODMAN, R. M., M.Sc., Ph.D., F.I.C., F.Inst.P., Horticultural Research Station, School of Agriculture, Cambridge.
1926 WOODWARD, R. C., M.A., B.Sc., Ph.D., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks. (Council 1931–2.)
1914 WORMALD, H., D.Sc., A.R.C.S., D.I.C., East Malling Research Station, East Malling, Kent. (Vice-President 1938–9; Council 1937–9.)
1936 WORSLEY, R. R. LE G., B.Sc., Ph.D., A.R.C.S., D.I.C., East African Agricultural Research Station, Amani, Tanganyika Territory, E. Africa.
1920 WORTLEY, E. J., C.M.G., O.B.E., F.C.S., Director of Agriculture, Port of Spain, Trinidad, B.W.I.
1940 WORTLEY, W. R. S., M.A., Ph.D., Plant Virus Research Station, School of Agriculture, Cambridge.
1941 COOK, C. Bannatyne Tree Service, 1273 Dufferin Street, Toronto, Canada (temporarily at No. 15 General Hospital, R.C.A.M.C., Hindhead).
1941 DARLINGTON, C. D., Ph.D., D.Sc., F.R.S., John Innes Horticultural Institution, Merton, London, S.W. 19.
1941 HILL, Miss E. H., B.Sc. (Hort.), Rothamsted Experimental Station, Harpenden, Herts.
1941 THOMAS, F. J. D., B.Sc., A.R.C.S., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks.
1941 WILSON, M. N. B., B.Sc. (Hort.), Technical Products Ltd., Lensbury Close, Kingston Road, Teddington, Middlesex.

PRINTED BY W. LEWIS, M.A., AT THE UNIVERSITY PRESS, CAMBRIDGE